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A TUBERCULOSIS SURVEY IN THE PAPAGO INDIAN AREA OF SOUTHERN ARIZONA

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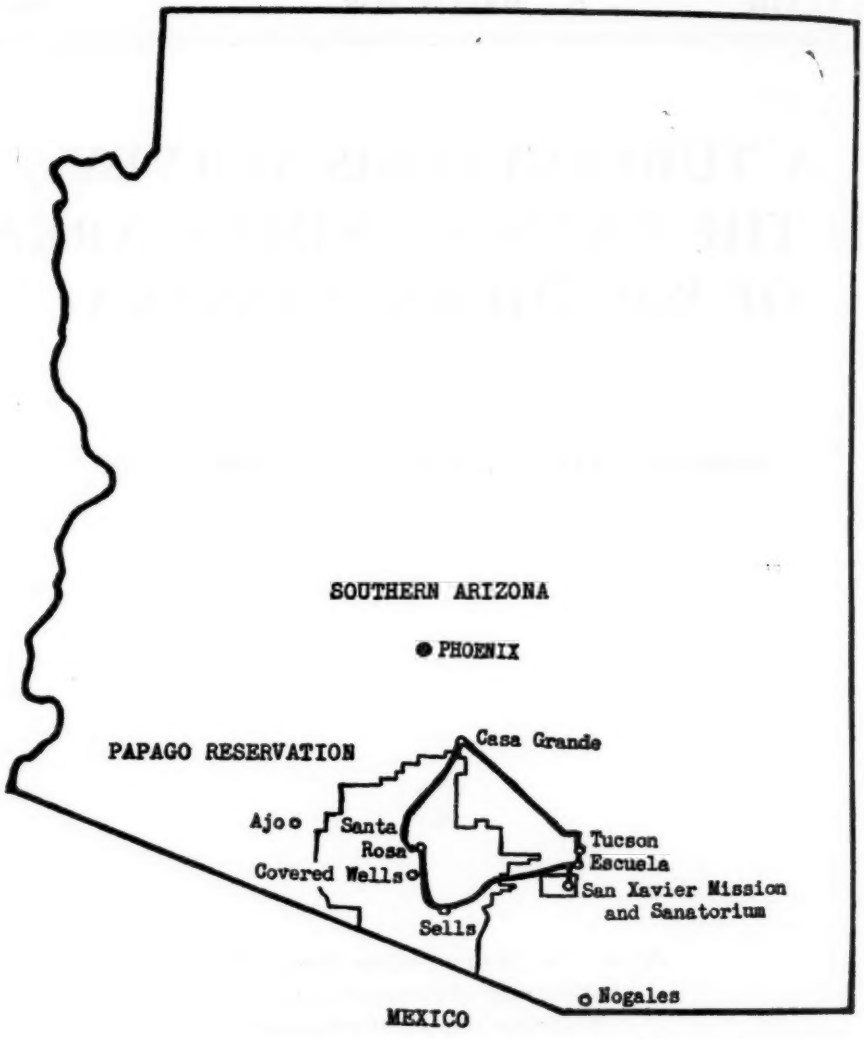

Fig. 1. Papago Reservation in Southern Arizona and Route Covered in Survey

A TUBERCULOSIS SURVEY IN THE PAPAGO INDIAN AREA OF SOUTHERN ARIZONA[1]

ESMOND R. LONG[2] AND H. W. HETHERINGTON[2]

Tuberculosis has long been recognized as a serious ill of the American Indian, but the extent of the problem has not been accurately defined. In recent years a number of surveys to determine its magnitude in different sections of the country have been made, and new studies on a larger scale are in progress at the present time. Ground-work for present appraisals was laid a dozen years ago in an investigation by a Committee of the National Tuberculosis Association, under the chairmanship of Dr. George M. Kober, which collected a large body of data on the prevalence of tuberculosis among the North American Indians, assembling records from the seventeenth to the twentieth century with a wealth of clinical observation from the early days of reservation control. The report of this committee, prepared with the active coöperation of the administrative and health officers of the Bureau of Indian Affairs, and published as a Senate Committee Print (1) in 1923, emphasized the unfavorable social and economic conditions of the Indian, rather than innate susceptibility, as the cause of the high tuberculosis rate, and recommended, as measures of control, addition to the medical personnel of the Indian Service, increase in sanatorium and hospital facilities, and the early initiation of measures to improve the economic state of the Indians.

In 1923 case-finding methods were far less developed than to-day. Ideas were not clear as to the course to follow, and the now common epidemiological procedures for determining the community incidence of tuberculosis were still in the stage of experiment and research. In recent years, following the standardization of tuberculin testing and X-ray programs, illuminating studies of the incidence of tuberculous infection and clinical tuberculosis have been made on several Indian reservations. It should be noted that Canadian physicians did admirable pioneer work in the modern investigation of tuberculosis in North

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American Indians, and Ferguson's (2) papers on tuberculosis among the Indians of the Great Plains are classics in their field. Succeeding studies in Canada are in progress, and Montgomery (3) and Walton (4) have recently published a highly informative analysis of the racial incidence of tuberculosis in Manitoba with special reference to the disease among Indians. In 1927 a study (5) was made of a limited number of Indians on the Klamath Reservation in Oregon, conducted by a staff drawn from a number of coöperating organizations, including voluntary and official Oregon health associations, the National Tuberculosis Association, the American Social Hygiene and Heart Associations and the United States Bureau of Indian Affairs. Nearly 5 per cent of the Indians examined had recognizable tuberculosis in some form, and in an equal number in addition there was reason to suspect latent tuberculosis. Recent studies on tuberculosis in Montana by a coöperating group of state public-health officers, U. S. Public Health Service surgeons, and local sanatorium officials and practising physicians, have made fairly clear the situation in that state. From these studies (6) (7) (8) it is apparent that the Indian tuberculosis mortality rate in Montana is far higher than the white rate, and that the infection rate is equally excessive, as shown by examination of Indian and white children attending the same schools. The view is advanced that a similar situation exists among Indians in other parts of the country. Studies on tuberculosis in the northern half of New Mexico and Arizona made by Dr. L. R. Jones, still in progress and not yet published, but communicated to us personally, confirm this view.

No survey is on record, however, of the incidence of tuberculosis among the Indians in the arid west, still further south, a region of moderate altitude, mild winters, high percentage of sunshine, and dry climate, long looked upon as salubrious for tuberculosis, and attractive for that reason to thousands of health-seekers from the eastern states. Advantage was accordingly taken of an opportunity to conduct a survey on the San Xavier and Papago Reservations of southern Arizona, in a region of about 5,000 population and 5,000 square miles' extent, consisting of two sections, one, the smaller, lying immediately west of the city of Tucson and the other, much larger, situated further west, bounded on the east and west, respectively, by the Baboquivari and Ajo ranges of mountains, and on the north and south by the Gila River Valley and the Mexican border.

ORGANIZATION OF SURVEY

The survey was organized for tuberculin testing and X-ray examination. Before the project was undertaken it was decided that in order to have statistical significance tuberculin tests must cover not less than 500 of the reservation population and include all age-groups. Actually about 530 Indians were tested, 512 tests being fully completed for record, with two doses of tuberculin administered if necessary. Practically all of those reacting positively were X-rayed. In preparation for the survey one of us (Long) was appointed Special Consultant in the Indian Service by the Commissioner of Indian Affairs. The Health department of the Service under Dr. J. G. Townsend, Director, and Dr. L. W. White, Assistant to the Director of Health, extended every facility in furthering the project. Local arrangements were made by Dr. R. H. Heterick, Medical Director of the area, Dr. Wm. A. Fahey, Superintendent of the San Xavier Sanatorium, Mr. T. B. Hall, Superintendent of the Sells Agency, and Dr. H. E. Montross, senior physician at Sells. The survey centered at the San Xavier Sanatorium. In the field and sanatorium work Miss Bertha Lips, field nurse, Miss Maude V. Pound, nurse in charge of the X-ray laboratory at San Xavier Sanatorium, Miss Newta M. Pearce, head nurse of the sanatorium, Mrs. Katheryn LeVan of the Sanatorium clerical staff, and Miss Erma Parr, field nurse in the Santa Rosa district, made the field operation of the survey rapid and successful. The superintending and teaching staffs of all the schools coöperated cordially and effectively. The group foremen in charge of Indian Emergency Conservation Work did quick and effective work in assembling men from the road construction projects. Finally, credit should also be given to the Indian bus-drivers who covered many miles to bring Indian groups to concentration points for tuberculin tests and to the San Xavier Sanatorium for X-ray examination.

The field work was carried out from March 11 to March 16, 1935. The route followed is shown in figure 1. Tuberculin tests were made at five points, at the Tucson Indian Training School (a Presbyterian Mission School at Escuela), San Xavier Mission (a Catholic mission school, which provided facilities for testing a number of Indians from the city of Tucson and men from the regional road construction camps, as well as the children of the mission school), San Xavier Sanatorium, Sells Agency and Santa Rosa. At Sells and Santa Rosa school children, workmen and workwomen were tested, and the Santa Rosa test included

school children from the neighboring village of Covered Wells. Such Indians as were not on the ground were assembled by bus. Figure 2 shows the gathering at Santa Rosa, figure 3 the type of Papago Village from which the Indians were largely assembled, and figure 4 a typical Papago home. Figures 5 and 6 illustrate representative groups of In-

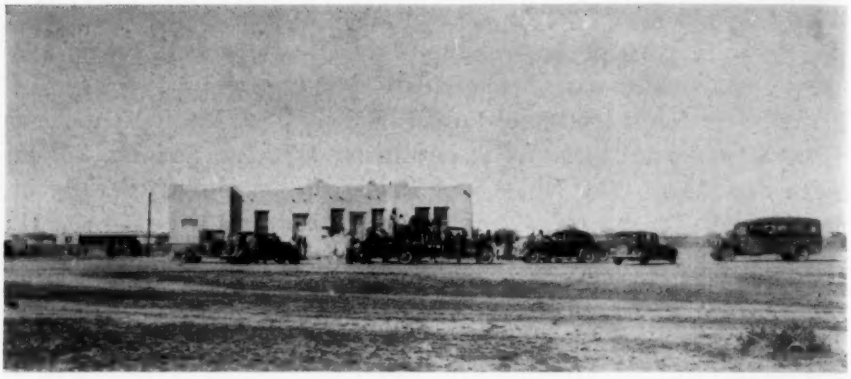

Fig. 2. Assembling Indians at Santa Rosa for Tuberculin Test

Fig. 3. Small Papago Village between Sells and Santa Rosa

dian school children and workmen; the pictures were taken at San Xavier Mission.

The tuberculin used was the Purified Protein Derivative developed by Seibert and recommended as a standard by the Committee on Medical Research of the National Tuberculosis Association (9). The recom-

mended procedure of administration was followed exactly, with second doses administered to those failing to react to the first dose.

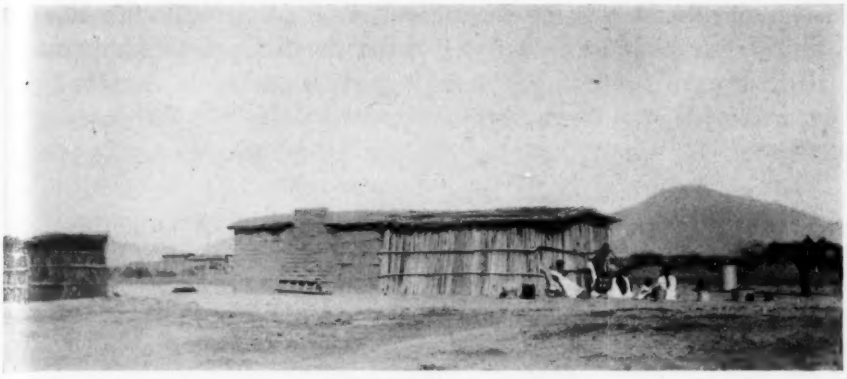

Fig. 4. Typical Papago Home on the Desert

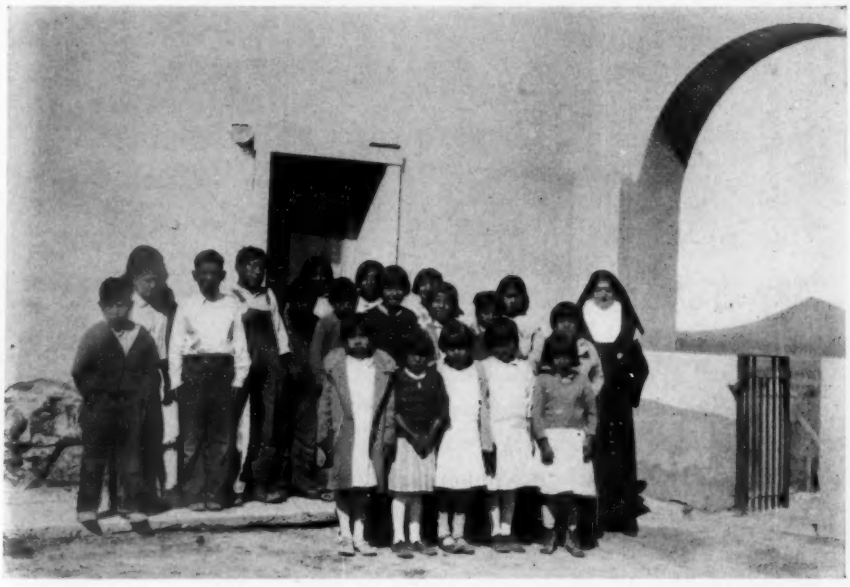

Fig. 5. Papago Indian Children at San Xavier Mission School

X-ray films of the positive reactors were made at the San Xavier Sanatorium. Distance and epidemic measles made it impossible to secure a film of every single reactor, but about 97 per cent of the reactors

were X-rayed in due course. In addition films were made in several cases in which the reaction was negative or the patient was unable to be present for tuberculin test. The films were read at the Henry Phipps Institute in Philadelphia by the authors, and in case of uncertainty or divergence of opinion were referred to Dr. F. Maurice McPhedran for final decision.

Fig. 6. Papago Workmen in Indian Emergency Conservation Work

COMPOSITION OF THE GROUP TESTED

It was originally hoped that a group totalling 500, with equal representation of five-year periods from birth to old age, could be studied. However, difficulties of transportation prevented the examination of more than a very few children under five years of age. Only five tests were completed, with the second dose. This was unfortunate because of the importance of examining children of this age-period in determining the extent of household infection. Otherwise the distribution of cases by age periods was fairly satisfactory. Children and adults were seen in approximately the proportion of three to two, and the adults represented age-periods from the third to the seventh decade. One deficiency was in the representation of women in the adult group.

About four-fifths of the adults tested were males. The majority of adults, both male and female, came from work camps of the Indian Emergency Conservation Work.

It is freely admitted that, in a total of 500, the figures for groups by age and location are too small to be of requisite volume for statistical conclusions, and also that examination of working Indians from the road camps hardly afforded a picture of the health conditions of Indians of the same age groups at home. Hence we are cautious in drawing conclusions and have limited our summarizing statements to the grosser aspects of the problem.

In this connection it should be noted that nearly half of all the children tested came from one school, the Presbyterian mission school at Escuela, and that this school was not exactly representative of the Papago area. One hundred and fifty-three boys and girls were tested there, of whom 59 were listed as Papagos and 69 as Pimas, a related Indian tribe, from the towns and reservations adjoining the Papago area, the remainder being Apaches, Mohaves and intermixtures, also from adjacent Indian areas. For this reason the results of tuberculin testing at Escuela and the rest of the Papago area are recorded separately as well as together (table 3).

In spite of these irregularities of representation, it is felt that the data collected afford ground for a reasonably accurate estimate of the seriousness of the tuberculosis problem in the Papago area and adjoining Indian country.

THE INCIDENCE OF INFECTION AS DETERMINED BY TUBERCULIN TEST

Standardized tuberculin, namely, Purified Protein Derivative in tablets, was used, and all injections were made intracutaneously in the forearm. Five hundred and twelve tests were completed, the second dose being administered 48 hours after the first if the first was negative.

Table 1 summarizes the results by age-periods, with notation of the severity of reactions. A high rate of infection, an increase in the incidence of positive reactions with advancing age, and the absence of an unduly high percentage of severe tuberculin reactions, are the significant facts shown in the table. The infection incidence is similar to that found in crowded sections of the large cities on the Atlantic seaboard or the poorer districts of Puerto Rico, but at least twice as high as that found in other regions in the west and middle west, in which localities surveys have recently been made by the authors with the standard

tuberculin. Unfortunately, there is no record available for comparison of the infection incidence in the white population neighboring the reservation. A high rate might be anticipated in the latter group also because of the local influx of health-seeking tuberculosis patients.

The level of severity of tuberculin reactions was about that seen in white patients in Philadelphia, and below that encountered in the colored race. This is in contrast to the findings of Aronson (10) among Indians

TABLE 1

Summarizing table of tuberculin reactions (PPD tuberculin), Papago and San Xavier Reservations, March 11–16, 1935

AGE-GROUP	NUMBER TESTED	POSITIVE TO 0.00002 MGM. PPD	POSITIVE TO 0.005 MGM. PPD	POSITIVE TO PPD	
				Total	Per cent
Under 5	5	1	0	1	20.0
5–9	82	45	6	51	62.2
10–14	186	125	16	141	75 8
15–19	80	50	10	60	75.0
20 or above	152	144	4	148	97.4
Totals.....	505	365	36	401	80.4
Average mm. dimensions of positive reactions.....		18.3 x 15.5 x 1.6 mm.	13.7 x 11.4 x 1.3 mm.		
INTENSITY OF REACTION			PERCENTAGE OF TOTAL POSITIVE TO 0.00002 MGM. PPD		
1+		110	30.1		
2+		148	40.6		
3+		96	26.3		
4+		11	3.0		
Totals.....		365	100.0		

in Isabella County, Michigan, where an exceptional incidence of severe tuberculin reactions occurred in a routine survey, but in agreement with the findings of Crouch in the Montana surveys (8). The average intensity of reaction was much like that noted by one of us in the slums of San Juan, where the rate is high and the disease is chronic and apparently of long standing in the community.

In table 2 figures are given showing the incidence of positive tuberculin reactions in the different groups tested, and in table 3 figures for

the school-children by sexes, with separate consideration of the mission school at Escuela. The figures for children in table 2 are significant when considered in the light of the social and economic status in the localities listed. The children in the Tucson Indian Training School at Escuela, a boarding-school, were in general in good physical condition and obviously receiving excellent housing and care. The children at San Xavier Mission, a day-school, were from a relatively crowded community of homes of low economic level, and were in visibly worse physical condition. The children from the schools at Sells, Covered Wells and Santa Rosa, also day-schools, while from families of generally low economic level, were from isolated villages remote from large centres of population like Tucson, and in the latter respect very different from

TABLE 2

Incidence of positive tuberculin reactions in different groups tested

	PLACE	TOTAL NUMBER	AVERAGE AGE IN YEARS	PER CENT POSITIVE
School children	Sells Agency	66	9.2	66.6
	San Xavier Mission	32	9.4	81.3
	Santa Rosa and Covered Wells	65	10.0	67.2
	Tucson Indian Training School at Escuela	153	13.8	68.0
Workmen and workwomen	Indian Emergency Conservation Work Camps	153	33.4	98.0
Patients	San Xavier Sanatorium	36	16.4	91.7

San Xavier Mission. Presumably this fact accounts, in part at least, for the lower rate in the former schools.

Another factor affecting the severity of tuberculin reactions may have entered in the case of Santa Rosa. The children here had just passed through a severe epidemic of measles. This may have led to a lowering or abolition of the tuberculin reaction in individual cases. The intensity of reaction was in general relatively low here, and, in a few cases in which there was reason to expect a positive reaction, the test was negative.

As noted, the incidence of infection was uniformly high in the working groups. As a matter of fact these groups included a few tuberculin-negative adolescents. Actually every adult 25 years of age or older was tuberculin positive.

While the numbers considered in table 3 are small, it is probably fair to draw certain distinctions. In the first place, in the reservation schools the incidence is slightly lower in girls than boys, the opposite of the fact found by Warner (8) in Montana Indians. Naturally, more extended observation would be needed to confirm this as a general rule for the region. Second, while the usual tendency for the positive tuberculin reaction to increase in frequency with age is seen in the reservation schools, this was not found at Escuela. Obviously the numbers in the group under 10 are too small to be of significance, but the figures for the 15–24 group, while also small, are probably large enough to permit a real distinction. Out of 16 girls in this age group tested on the reservation, including 7 in the day-schools, only 2 were negative. Among 34 girls in this group at Escuela 15 were negative. It was at first thought that the discrepancy was due to difference in tribal make-up, since the Tucson Indian Training School includes many Pimas from the Sacaton region, where Dr. Jones (unpublished observations communicated to us personally) has found a relatively low infection rate. Examination of the protocols, however, showed no significant difference in the infection rate among Pimas and Papagos at Escuela. The explanation seemed most logically to lie in the fact made known to us by Dr. M. L. Girton, Superintendent of the Indian Training School at Escuela, that the older girls had been with the school many years, with trips home only once a year, and even then in the summer when life was largely outdoors, and

TABLE 3

Incidence of positive tuberculin reaction in school children

	AGE-GROUP	NUMBER TESTED		POSITIVE TO TUBERCULIN				TOTALS		
				Number		Per cent				
		Boys	Girls	Boys	Girls	Boys	Girls	Number	Positive	Per cent
San Xavier Mission, Sells Agency, Covered Wells and Santa Rosa schools	Under 10	38	36	23	21	60.5	58.3	74	44	59.4
	10–14	34	44	26	35	76.5	79.5	78	61	78.3
	15–24	3	7	3	6	100.0	85.7	10	9	90.0
Tucson Indian Training School at Escuela	Under 10	4	4	3	2	75.0	50.0	8	5	62.5
	10–14	43	49	28	38	65.1	77.5	92	66	71.7
	15–24	19	34	14	19	73.7	55.9	53	33	64.1
All schools combined	Under 10	42	40	26	23	61.9	57.5	82	49	59.8
	10–14	77	93	54	73	70.1	78.5	170	127	74.7
	15–24	22	41	17	25	77.3	61.0	63	42	66.6

crowding and corresponding opportunity for contagion at a minimum. The survey indicated that opportunity for spread of infection at Escuela must have been small. While a few cases of active tuberculosis were discovered (3 altogether, 2 of them advanced), the hygienic standards at the school were high and the danger of infection-spread unconsciously minimized.

Some insight into the familial incidence of tuberculosis on the Papago and San Xavier Reservations was obtained from the grouping by households recorded for the children at the day schools. Families wholly positive and a few small families wholly negative to tuberculin were discovered. But, in the communities studied, where the general infection incidence was so high, study of the tuberculin reaction by families in the time available did not prove a way of locating cases of open tuberculosis.

THE INCIDENCE OF PULMONARY TUBERCULOSIS AS SHOWN BY X-RAY EXAMINATION

The results of the X-ray examinations are given in table 4. As noted above, X-ray films were made of 97 per cent of the positive reactors and a certain number of tuberculin-negative and untested Indians as well. In all, 428 films were studied.

The classification used was very simple, chief divisions being adult and childhood types of tuberculosis. The former was subdivided into the clinical groups of far advanced, moderately advanced and minimal tuberculosis, and a fourth group in which consideration of the extent and appearance of the infiltration and the age of the patient led us to believe that the lesion was of long standing and unlikely to progress. The childhood type was divided into presumably clinically active cases and cases with calcified lesions. A few cases in which tuberculosis was merely suspected were recorded for both adult and childhood types.

Eleven cases classified as far advanced were discovered. Six of these were in the San Xavier Sanatorium, two were in the village neighboring San Xavier Mission, and one each was found at Escuela, Sells Agency and the village of Covered Wells. Four of the patients (all in the sanatorium) were girls or young women. The age range for the whole group was 13 to 60 years (13, 13, 15, 16, 18, 19, 21, 22, 25, 33 and 60 years).

There were 17 cases classified as moderately advanced. Nine of these were in the sanatorium. Three (including one case of childhood type) were found at Sells Agency, two were in the village of Santa Rosa, and

TABLE 4

*Summarizing table of chest X-ray examinations of Southern Arizona Indians including patients in San Xavier Sanatorium**

	AGE-GROUP							TOTAL
	Under 5	5–9	10–14	15–19	20–24	25 or above	Not given	
Adult-type tuberculosis								
Far advanced			2	4	2	3		11
Moderately advanced			3	4	3	5		15
Minimal			5	1	2	12		20
Minimal; of no apparent clinical significance					1	10		11
Suspected			3	1	1			5
Childhood-type tuberculosis								
Active		1	1					2
Apparently inactive, with calcified lesions		12	14	8	6	6	2	48
Suspected		1		1				2
Negative	5	41†	109	42	22	84	11	314
Total	5	55	137	61	37	120	13	428

*Summarizing table of chest X-ray examinations of Southern Arizona Indians excluding patients in San Xavier Sanatorium**

	AGE-GROUP							TOTAL
	Under 5	5–9	10–14	15–19	20–24	25 or above	Not given	
Adult-type tuberculosis								
Far advanced			1	1		3		5
Moderately advanced			2	2	1	2		7
Minimal			3	1	2	11		17
Minimal; of no apparent clinical significance					1	10		11
Suspected			1	1	1			3
Childhood-type tuberculosis								
Active			1					1
Apparently inactive, with calcified lesions		11	13	7	6	6	2	45
Suspected		1		1				2
Negative	5	38	106	40	20	83	11	303
Total	5	50	127	53	31	115	13	394

* 428 subjects were examined by X-ray. These included 393 Indians positive to tuberculin and 35 either negative to tuberculin or untested. 34 subjects were patients in the San Xavier Sanatorium.

† One boy without demonstrable pulmonary tuberculosis had tuberculosis of the hip.

one each was discovered at Escuela and the village at San Xavier Mission. One also was found in the city of Tucson close to San Xavier Mission. The cases were about equally divided between males and females, and the age-range was 5 to 54 years (5, 12, 13, 14, 14, 16, 17, 19, 19, 21, 21, 23, 25, 25, 34, 45 and 54 years) .

Twenty Indians were found with tuberculous infiltration similar to that of the moderately and far advanced cases but minimal in extent. Three were in the sanatorium, seven at Santa Rosa, five in the village at San Xavier Mission, three at Sells Agency, and one each at Escuela and Covered Wells. Fifteen of the twenty were males. Ages ranged from 10 to 60 (10, 11, 11, 11, 14, 15, 21, 23, 25, 25, 28, 28, 28, 31, 40, 40, 45, 45, 50 and 60 years).

In 11 cases there was X-ray evidence of apical scars interpreted as minimal tuberculosis apparently of long standing and inactive. All of the group were adults, the youngest being 21 years old and the oldest 65 (21, 31, 35, 35, 38, 40, 41, 44, 47, 56 and 65 years). Only two were females. Most of the group were from the construction camps working on the roads under the Indian Emergency Conservation Work.

In the group in which the films did not permit a definite diagnosis, but shadows existed that might represent minimal tuberculosis, were 3 girls and 2 boys (ages 11, 12, 12, 19, 22 years). One boy (12 years) and one girl (11 years) were in the sanatorium. Two of the remainder were at Escuela and one in the village of San Xavier.

While, as the figures above indicate, a considerable number (20) of cases of early adult-type tuberculosis were discovered, the survey brought to light only two cases (boy, 5 years, and girl, 14 years) definitely considered as having childhood-type pulmonary infiltration in the active stage. Two cases (girl, 8 years, and boy, 17 years), considered as probably childhood-type tuberculosis with involvement of the tracheobronchial lymph nodes, were found.

In contrast, the number of cases with calcified lesions of the childhood type, and no other evidence of tuberculosis, was considerable, 48 in 428 films. The table indicates their distribution by age-groups. An additional 16 instances of calcified lesions of childhood type were found in combination, thirteen with definite apical infiltration and three with suspected apical infiltration. These lesions are discussed below.

CORRELATION OF X-RAY RESULTS AND TUBERCULIN REACTIONS

Facts of general significance developed in the comparison of X-ray findings and intensity of tuberculin reaction. The point of most impor-

tance is the fact that of 48 cases of tuberculosis regarded as clinically significant all but one reacted to the first dose of the purified tuberculin (0.000,02 mgm.). The list included 10 out of 11 cases of far-advanced adult type, all 15 of moderately advanced adult type, all 20 of minimal extent and adult type, and the two cases diagnosed as active childhood-type tuberculosis. In other words, had the first dose only been applied in the 428 Indians subjected to X-ray examination, only one case of active tuberculosis would have been missed. This case was one of far-advanced disease. The second dose of tuberculin (250 times the strength of the first) elicited a positive reaction in this case. It is a common observation that the sensitiveness of patients with far-advanced tuberculosis is apt to be of low degree. In cases of this severity the reaction may even be negative. It is probable that such patients are completely desensitized by the overwhelming disease in their own tissues. The disease is so advanced in these cases, however, that its nature is usually obvious. All of the 11 cases, diagnosed as adult type of minimal extent and apparently inactive, responded to the first dose of tuberculin.

Again, the great majority of the cases of childhood-type tuberculosis, with calcified lesions and no evidence of adult-type disease, reacted to the first dose, namely, 43 out of 48. Three of those failing to react responded to the second dose (girls, 17, 16 and 12 years of age), but two remained negative even to the larger dose (one boy and one girl, each 7 years of age). It is well known from general experience that occasionally patients with well calcified lesions fail to react to tuberculin. It is commonly believed that this waning of sensitiveness in these cases signifies an unusually thorough healing of the lesion, but data adequate for sure conclusions are lacking. In this case, however, another factor may have entered. Of the two nonreacting children with calcified, presumably tuberculous lesions, one had just recovered from whooping cough and the other from a severe attack of measles. Of the three reacting only to the second dose one was convalescent from measles apparently complicated by bronchopneumonia. It is well known that these acute febrile diseases reduce tuberculin allergy.

Altogether it is apparent that the very small dose of tuberculin was effective in calling attention to the overwhelming majority of cases of clinical significance located in this survey. This may perhaps be an answer to that large body of investigators who feel that all practical purposes will be served by using one dose of tuberculin and dispensing

with a second larger dose. Many of these investigators would compromise on an intermediate dose, which will detect a number of cases missed by the standard small first dose. This, of course, would increase the severity of the reaction in those who do respond to the first test. In this survey a certain number of the three-plus reactions would have become four plus, and the reactions already four-plus would have been still more severe. It is a matter of individual judgment how far one may go in this direction; it must be recognized that an excessive number of severe reactions does harm to the cause of tuberculin-testing, by creating opposition in pupils and parents. In this particular instance it should be noted that the percentage of very severe reactions obtained was not much greater than that generally found among whites in regions where the infection incidence is high. It was distinctly less than the percentage we commonly find in the colored population of corresponding age in the Philadelphia area. As noted above, Aronson (10) found a group of Indians in Michigan displaying exceptionally high sensitiveness, but the results here secured and those obtained by others indicate that high tuberculin allergy is not a general rule for Indians, as it appears to be in our colored population.

DISCUSSION OF THE INCIDENCE OF TUBERCULOUS LESIONS OF THE CHILDHOOD TYPE

Sixty-four instances of calcified primary lesions (either pulmonary nodules or tracheobronchial lymph node lesions or both), with or without adult-type tuberculosis, were demonstrated by X-ray examination. This number (12.5 per cent of the 518 subjects examined with X-ray, tuberculin or both in the survey) represents only a portion of the lesions of this type that might have been found by a more complete examination. No attempt was made to verify suspected lesions of this nature that were not definite on one examination, and films in the oblique position to show calcification at the interbronchial angle and about the trachea were not taken. Again, the majority of tuberculin-negative subjects were not examined by X-ray; a few cases of primary calcifications in subjects insensitive to tuberculin may have been present in them and not demonstrated.

Most instances of calcified lesions of the tracheobronchial lymph nodes, as Opie and McPhedran (11) have pointed out, occur in children who have been exposed to intimate contact with tuberculosis. Analyzing families of which a member had sputum-positive tuberculosis,

they found that 47.5 per cent of children of the age-group 5 to 9 and 59.8 per cent of children of the age-group 10 to 14 had calcified tuberculous foci. In the Indians at present under consideration 12 of 89 or 13.5 per cent of children aged 5 to 9, and 20 of 170 or 11.8 per cent of children aged 10 to 14 had similar lesions. Our figures for this type of lesion among Southern Arizona Indians approximate the results obtained here and elsewhere in similar surveys of white people not selected on a contact basis, a finding somewhat surprising when the high tuberculosis morbidity and mortality of the Indians are considered.

Thirteen of the 64 instances of calcified tuberculous foci occurred in the 58 individuals who exhibited apical infiltration, and are not shown in table 4. This incidence, namely, 22.4 per cent, closely approximates the findings for the same association of lesions in high-school students in Philadelphia (12). Since tuberculosis is characteristically a household disease, the frequency of association of the two types of lesion may be explained by the greater opportunity for infection and reinfection from a new source in adult life.

Only two instances of pulmonary infiltration of presumably childhood-type tuberculosis were found. One was in the sanatorium. The lesion in the other (depicted in plate 1 as M.A.-8) consisted of a wedge-shaped consolidation sharply limited below by the interlobar line between the right upper and middle lobes. It appeared to conform to either type B or type C of McPhedran's classification (13), but there was no evidence of either caseous or calcified tracheobronchial lymph nodes. In two instances (table 4) enlargement of hilum nodes (presumably in part caseous) was suspected, but no definite decision could be made from the single film under consideration. The above incidence of childhood-type tuberculosis does not bear out the commonly accepted belief that lesions of this nature are frequent among Indians and that they are responsible for an appreciable percentage of clinical cases of tuberculosis.

PATHOLOGICAL TYPE OF TUBERCULOSIS IN THE PAPAGO INDIANS

The following facts may be recalled for consideration of the type of tuberculosis found in this survey among Indians. The adult type of pulmonary tuberculosis usually pursues a chronic course beginning at or near the apex of the lung, spreading downward and producing cavity-formation and fibrosis, the so-called productive form of the disease. Less often extensive disease, often nonapical, may develop, rapidly reaching the moderately advanced or even far-advanced stage with

slight formation of fibrous tissue, the so-called exudative form. Tuberculous lesions in which there is little evidence of fibrosis on X-ray examination are common among Negroes, less common among whites. Their X-ray appearance is homogeneous clouding or flocculent spots, frequently confluent. Productive lesions, on the other hand, are in large part sharply defined and strand-like.

Among the 13 instances of moderately and far-advanced tuberculosis depicted in the diagrams on plate 1 only 2 cases showed little or no evidence of chronicity. One of these (M.A.-8) is the case of childhood type already cited above. The other (M.A.-1) occurred in a girl of 12 years. Another case (F.A.-1) showed a dense shadow over the upper third of the lung, but the displacement of the trachea, and the strand-like appearance in the middle third of the right lung and the upper two thirds of the left lung are in favor of the existence of chronic disease, despite the subject's age of thirteen years.

A division of the 17 minimal lesions shown in plate 2, on the same basis seems to us impractical. Most of the lesions in the minimal group were partially strand-like in character. In the others the limited extent of the lesion suggests that it is comparatively recent or that it is being held in check by the resisting forces of the body.

In the sanatorium there were 18 patients with pulmonary tuberculosis, not discovered for the first time by the survey, but previously diagnosed because of ill health. One of these patients was a child of 5 years with tuberculosis of the childhood type, moderately advanced in extent. Another patient, aged 18 years, had a moderately advanced lesion with a cavity about 2 cm. in diameter, in the middle third of the right lung. This lesion was exudative in type and the patient died 5 months later. These two cases resemble tuberculosis as it occurs commonly in the Negro. A third patient had a dense shadow obscuring the left lung field, presumably in part due to thickened pleura, and a dense flocculent shadow at the base of the right lung due to infiltration. Although the X-ray appearance did not suggest chronicity, the patient had already been confined to bed for 18 months with tuberculosis. The extent, distribution and other characteristics of the lesions in the remaining 15 patients resembled the more chronic, productive type of tuberculosis commonly seen in white patients.

Summing up, taking into account the character and extent of lesions, as well as the scarcity of significant progressive disease of the childhood type, we feel that pulmonary tuberculosis among the Indians studied

PLATE 1

Pulmonary Tuberculosis among Southern Arizona Indians

Advanced Lesions

F.A.-1. S. R., Papago, Covered Wells, male, 13 years, tuberculin 3+, far-advanced bilateral pulmonary tuberculosis

F.A.-2. A. A., Papago, Escuela, male, 15 years, tuberculin 2+, far-advanced bilateral pulmonary tuberculosis

F.A.-3. F. R., Papago, San Xavier, male, 25 years, tuberculin 2+, far-advanced bilateral pulmonary tuberculosis

F.A.-4. A. C., Papago, San Xavier, male, 33 years, tuberculin 3+, far-advanced bilateral pulmonary tuberculosis

F.A.-5. T. P., Papago, Sells, male, 60 years, tuberculin 2+, far-advanced bilateral pulmonary tuberculosis and calcified primary tuberculous lesion, right

M.A.-1. L. G., Papago, Santa Rosa, female, 12 years, tuberculin 2+, moderately advanced pulmonary tuberculosis right and probably left

M.A.-2. L. F., Papago, Santa Rosa, male, 14 years, tuberculin 1+, moderately advanced bilateral pulmonary tuberculosis

M.A.-3. H. H., Pima, Escuela, male, 16 years, tuberculin 3+, moderately advanced bilateral pulmonary tuberculosis

M.A.-4. E. F., Papago, Sells, male, 17 years, tuberculin 3+, moderately advanced bilateral pulmonary tuberculosis

M.A.-5. T. L., Papago, Sells, male, 21 years, tuberculin 1+, moderately advanced pulmonary tuberculosis, right

M.A.-6. J. A., Papago, San Xavier (Tucson), female, 45 years, not tested with tuberculin, moderately advanced bilateral pulmonary tuberculosis and calcified primary tuberculous lesion, right

M.A.-7. C. M., Papago, San Xavier, male, 54 years, tuberculin 3+, moderately advanced bilateral pulmonary tuberculosis

M.A.-8. C. M., Papago, Sells, female, 14 years, tuberculin 2+, moderately advanced pulmonary tuberculosis, right, childhood type

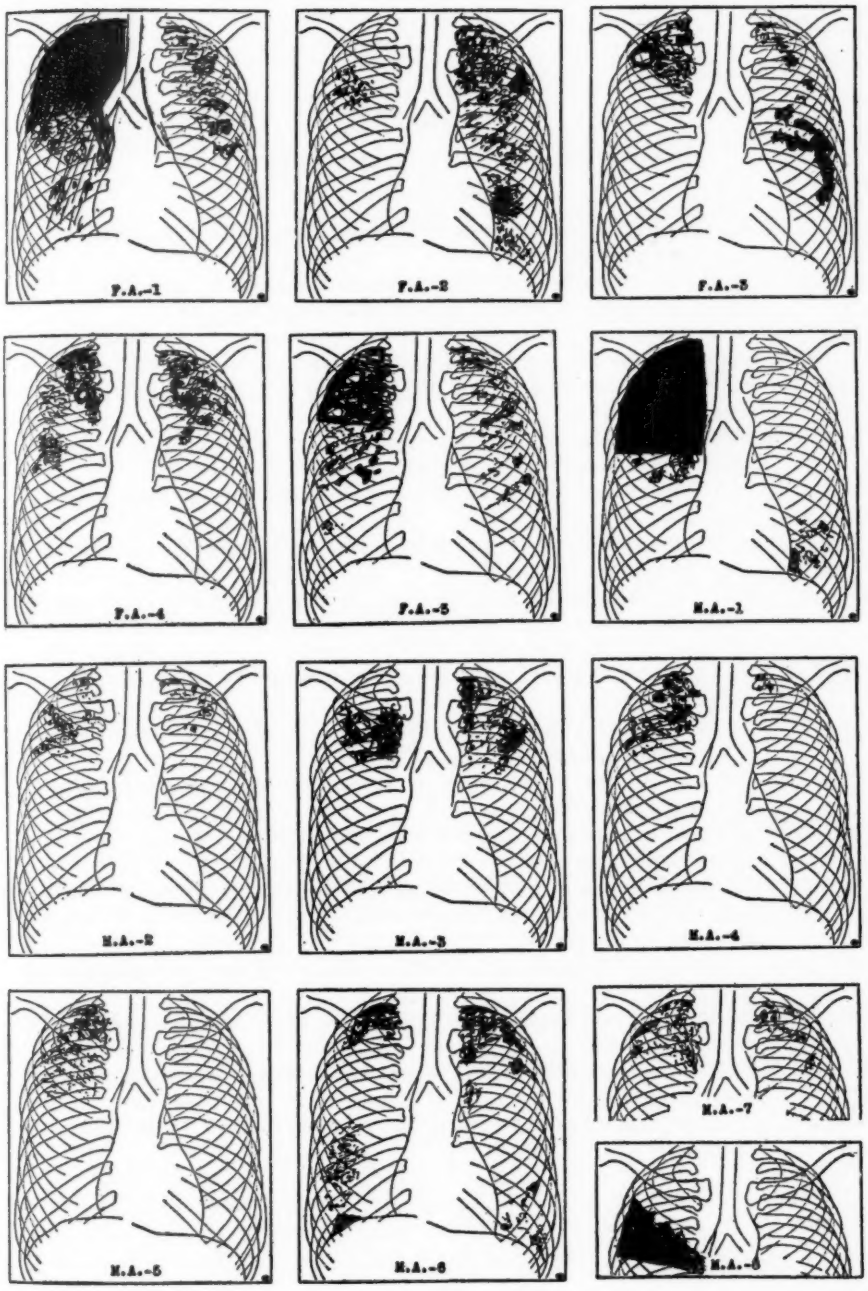

PLATE 1

PLATE 2

Pulmonary Tuberculosis among Southern Arizona Indians

Minimal Lesions

Min.-1. L. R., Papago, Covered Wells, male, 11 years, tuberculin 2+, minimal bilateral pulmonary tuberculosis

Min.-2. A. P., Papago, Santa Rosa, female, 11 years, tuberculin 2+, minimal pulmonary tuberculosis right, and calcified primary tuberculous lesion, left

Min.-3. M. N., Papago, San Xavier, female, 11 years, tuberculin 3+, minimal bilateral pulmonary tuberculosis and calcified primary tuberculous lesion, left

Min.-4. D. S., Papago, Santa Rosa, female, 15 years, tuberculin 4+, minimal pulmonary tuberculosis, right

Min.-5. W. G., Pima, Escuela, male, 21 years, tuberculin 2+, minimal pulmonary tuberculosis, left (left tuberculous pleurisy with effusion 10 days later)

Min.-6. J. F., Papago, Santa Rosa, male, 23 years, tuberculin 2+, minimal pulmonary tuberculosis, left

Min.-7. P. C., Papago, Sells, male, 25 years, tuberculin 1+, minimal bilateral pulmonary tuberculosis

Min.-8. L. E., Papago, San Xavier, male, 28 years, tuberculin 3+, minimal bilateral pulmonary tuberculosis (with extensive calcification, left) and calcified primary tuberculous lesions, right and left

Min.-9. J. O., Papago, San Xavier, male, 28 years, tuberculin 2+, minimal pulmonary tuberculosis, right

Min.-10. F. R., Papago, San Xavier, male, 28 years, tuberculin 3+, minimal bilateral pulmonary tuberculosis

Min.-11. J. M., Papago, San Xavier, male, 31 years, tuberculin 1+, minimal bilateral pulmonary tuberculosis

Min.-12. J. J., Papago, Santa Rosa, male, 40 years, tuberculin 3+, minimal bilateral pulmonary tuberculosis

Min.-13. J. K., Papago, Santa Rosa, male, 40 years, tuberculin 2+, minimal bilateral pulmonary tuberculosis (with calcification on right) and calcified primary tuberculous lesion left

Min.-14. V. J., Papago, Sells, female, 45 years, tuberculin 2+, minimal bilateral pulmonary tuberculosis

Min.-15. J. R., Papago, Santa Rosa, male, 45 years, tuberculin 2+, minimal bilateral pulmonary tuberculosis

Min.-16. S. F., Papago, Santa Rosa, male, 50 years, tuberculin 2+, minimal pulmonary tuberculosis right

Min.-17. L. R., Papago, San Xavier, 60 years, tuberculin 2+, minimal bilateral pulmonary tuberculosis

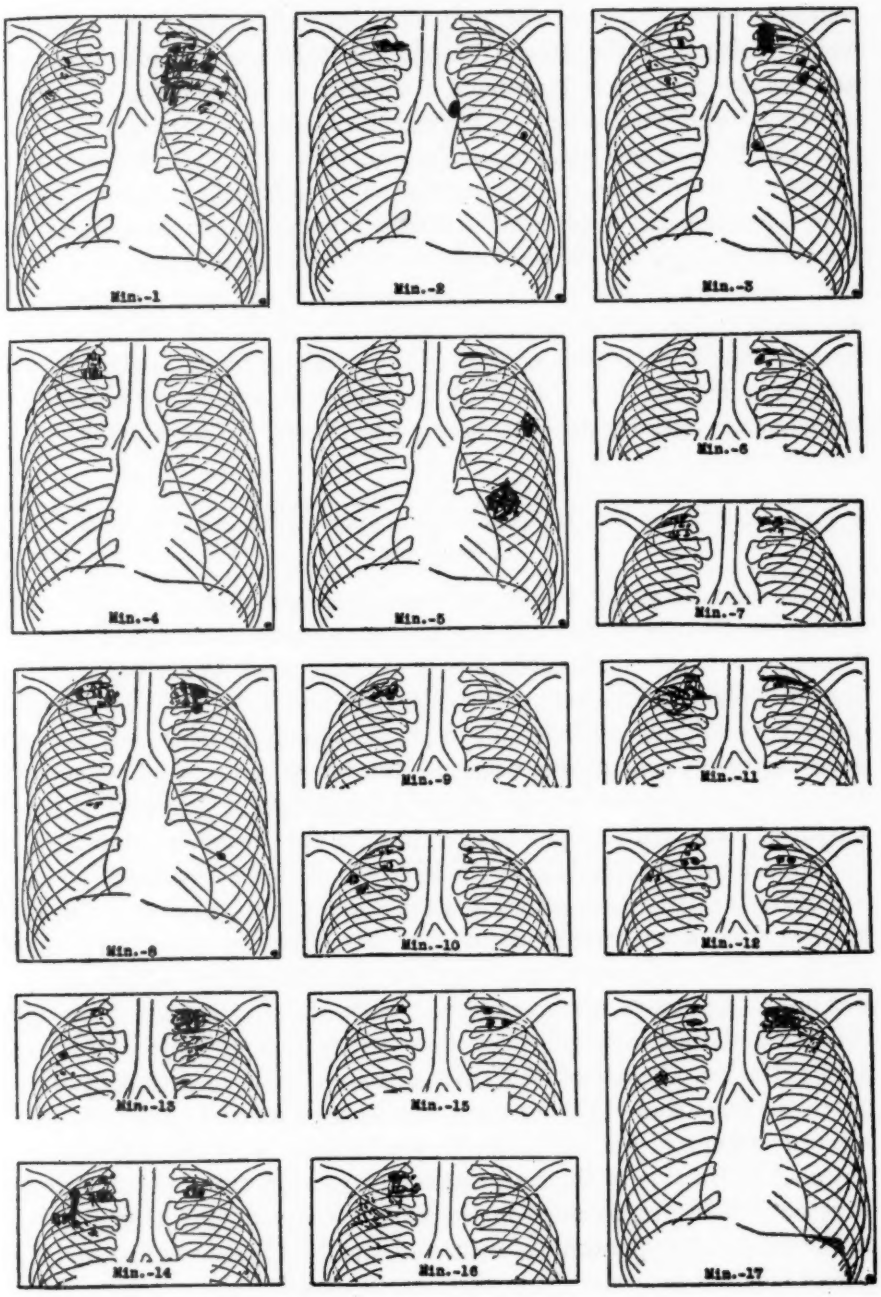
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PLATE 2

conforms more closely to the type of pulmonary tuberculosis seen in white patients at the Henry Phipps Institute than to the massive and rapidly progressive type more often seen in Negro patients. This conclusion conforms with the statement of Korns and Atwater (14) that the Indians in Cattaraugus County, New York, seemed to show considerable resistance to tuberculosis. It is only fair to admit, however, that the cases of tuberculosis in the population not seen either in the sanatorium or the survey might have been of a more disabling type than those seen in our survey, and might have been found on X-ray examination to be of a more acute, exudative type like that seen in the colored population of this country. It should be noted however that there was nothing in the films of the sanatorium patients, who were brought from outlying districts because of illness, to indicate that this was the case.

ESTIMATION OF THE INCIDENCE OF ACTIVE TUBERCULOSIS IN THE PAPAGO AREA

If it could be made, a close estimate of the amount of tuberculosis among the Indians in the entire area covered, on the basis of the results from the five hundred examined, would be a useful addition to our knowledge of Indian tuberculosis. Had the group seen been representative of the population as a whole, in age, sex, habits, occupation, health and opportunity for tuberculosis contact, such an estimate could be made. It would only be necessary to multiply the figures for the studied five hundred by ten to arrive at an approximation for the whole reservation, the population of which is in the neighborhood of five thousand.

Obviously, perfect representation was impossible to obtain. In all surveys there must be some element of selection. In the present case the basis of selection was, to a large extent, availability. The survey was limited to groups most readily accessible, made up chiefly of school-children and Indians working on construction projects under the relief administration. Three of the most serious defects in representation lay in the facts that (1) those known by themselves to be sick were not reached (except in the study of the sanatorium, in which presumably but a small fraction of the reservation sick were present), (2) the sexes were not equally represented in the adult group, which was made up, to the extent of 80 per cent, of men, and, finally, (3) the number of pre-school children seen was disproportionately small.

The figures for school-children are perhaps the most significant, but

here too selection could not be avoided. Five groups of school-children were examined, about half of them from four day-schools and the other half from a single boarding-school. There is reason to believe that the schools represented the two extremes in opportunity for contact with tuberculosis. In the day-schools were a certain number of children in nightly contact with sick adults in the homes. In the large boarding-school at Escuela, on the other hand, the children were for more than eight months of the year out of contact with their families. And in this connection it is to be noted that the incidence of clinical tuberculosis was relatively low in this group, in spite of the fact that a relatively high proportion of the group were in the susceptible adolescent age (see table 3). Another defect in representation was inherent in the composition of the Tucson Indian Training School at Escuela. It was not strictly representative of the Papago area in which it was geographically located, as the pupil body included many Pimas from the region adjoining the Papago area, and a few Indians from more distant tribes.

The facts just cited indicate a selection largely in favor of a low incidence of tuberculosis, for (1) the group studied was limited to accessible subjects (school children and adult workers) believed to be healthy, (2) a relatively small number of women in the third decade, so prolific for tuberculosis in the rest of the American female population, were seen, and (3) about half of the school-children examined, or 30 per cent of the entire surveyed group, were pupils of a boarding-school in which a good deal of home contact was eliminated.

On the other hand, the absence of any considerable number of pre-school children from the survey was a factor of selection toward an abnormally high incidence of pulmonary tuberculosis, since this disease is relatively rare in the first decade of life. Obviously the inclusion of known tuberculosis patients in the small San Xavier Sanatorium operated in the same direction, while their omission would operate in the opposite one, as the sanatorium patients were derived largely from homes in the area studied. The involved statistical computations for correction on this score were not attempted, the figures for the total group seen, and the group minus the sanatorium patients, being presented separately for the consideration of those interested.

On the whole, while it is impossible to determine the exact balance of the factors operating in the two directions, it seems probable to us that the selection involved in the survey led to the discovery of less rather than more than the actual percentage of cases of pulmonary

tuberculosis per unit of population. In spite of this selection an astonishingly large amount of clinical pulmonary tuberculosis was discovered. Thirty cases of far-advanced, moderately advanced or minimal tuberculosis were found, outside of the sanatorium, in the 482 subjects examined by tuberculin test and X-ray. The reservation population for 1934–1935 in the areas studied was about 5,200. If the 482 subjects were representative of the population at large, the calculated figure for the reservation population at large would be 322, and the case incidence 62 per thousand. Actually, as we have indicated, from the selection involved there is reason to believe that a somewhat higher proportion of tuberculosis occurred in the Indians not in the survey than in the group actually seen. On this basis we believe the actual number of cases exceeded 62 per thousand.

It is worth noting at this point that among the 30 cases discovered thirteen were moderately or far advanced, probably with positive sputum. If the same proportion of cases held in the rest of the population, there would be, following the admittedly questionable case-rate figure calculated above, about 27 people per thousand on the reservation with sputum positive for tubercle bacilli.

TUBERCULOSIS MORTALITY AMONG THE PAPAGOS

The official returns for tuberculosis in the Papago area, as obtained from the Office of Indian Affairs in Washington, were 10 deaths in a population of 5,146 in the year 1933–1934 and 13 deaths in 5,147 in 1934–1935. On the basis of these figures the uncorrected mortality for tuberculosis would be about 223 per 100,000. There is every reason to believe the tuberculosis mortality is much higher than this. Inaccessibility of patients and the physical limitations of a small medical staff prevent medical attendance on more than a small fraction of the tuberculous sick on the reservation. The deaths recorded as from tuberculosis are those in patients seen by the medical personnel and those coming to the attention of the field nurses, where the symptomatology was definitely such as to indicate pulmonary tuberculosis as the probable cause of death. Many deaths from tuberculosis must remain unknown.

The figures for case incidence presented in this paper probably permit a closer approximation of the tuberculosis death-rate. Admittedly, at the most, the approximation is very rough. It is commonly held that in white communities annual deaths from tuberculosis and cases discoverable by tuberculin-X-ray survey are in the proportion of about 1 to 10. In this survey 30 cases of clinical tuberculosis and 11 cases of

nonclinical tuberculosis, or 41 in all, were found in about 500 subjects outside of the sanatorium. If the annual death-rate is in the proportion of one death to ten such cases in Indians also, it would be calculated at 8.2 per thousand or 820 per hundred thousand, provided the group seen were representative of the whole population. As we have seen, this was not exactly the case. But the analysis given above, leading to the view that figures based on the survey are minimal figures, would apply equally to the calculated death-rate. It seems likely to us, therefore, that more than 8 per thousand die each year from pulmonary tuberculosis. This is about four times the reported death-rate, but not greatly different from the recorded census rate for the Papagos twenty-eight years ago, listed in Hrdlicka's compilation (15), and much the same as the rate listed for Montana.

NONPULMONARY TUBERCULOSIS

No attempt was made to determine the incidence of nonpulmonary tuberculosis. Two cases of bone tuberculosis were seen. One of these, a case of tuberculosis of the hip was in the sanatorium, and a case of healed tuberculosis of the spine was seen in the group tested at Sells. In neither case was there unmistakable pulmonary tuberculosis. Draining cervical lymph nodes were not seen, nor was any conspicuous tendency to enlargement of these lymph nodes observed. On the whole, the impression was obtained that the incidence of nonpulmonary tuberculosis in the group examined was not significantly different from that to be found in white people of the same sex and age in this country in a similar random selection.

SUMMARY

A tuberculosis survey was carried out on about 530 Indians on the Papago and San Xavier Reservations in Southern Arizona, including school pupils and adults in the proportion of about three to two. The children were from four day-schools and one boarding-school, and about equally divided between the sexes. The adults were largely from labor projects of the Indian Emergency Conservation Work, and were chiefly men.

Eighty per cent of the Indians tested were positive to tuberculin (Purified Protein Derivative of tuberculin in tablet form). By age-groups the incidence was as follows: 5–9 years, 62 per cent; 10–14 years, 76 per cent; 15–19 years, 75 per cent; 20 years or above, 97 per cent.

Presumptive evidence of family contact infection was apparent from

the compilation of tuberculin reactions by households. Also, a relatively low rate of positive tuberculin reactions in children at one large boarding-school, who were away from home most of the year, was further evidence of the part played by infection in the home. Lack of time and incompleteness of data, however, prevented use of family records for locating cases of open disease.

About 97 per cent of those positive to tuberculin were X-rayed in the San Xavier Sanatorium. In addition, a few Indians, negative to tuberculin or untested, were also X-rayed. Fifty-seven cases of adult-type tuberculosis were found, divided in clinical significance as follows: 11 far advanced (including 6 in the sanatorium), 15 moderately advanced (including 8 in the sanatorium), 20 minimal (including 3 in the sanatorium), and 11 inactive. Two cases of moderately advanced pulmonary tuberculosis of childhood type were diagnosed (including one in the sanatorium), and in two instances tuberculous tracheobronchial lymphadenitis was suspected. The 29 cases of presumably active adult-type tuberculosis, together with the one case of moderately advanced childhood-type tuberculosis, made up 6.2 per cent of 482 subjects outside of the sanatorium examined by tuberculin test and X-ray. The 13 cases of far or moderately advanced tuberculosis outside of the sanatorium (including the one childhood-type case) made up 2.7 per cent of the 482 subjects. These 13 cases were presumably sputum-positive and responsible for further spread of the disease in the community.

Calcification of pulmonary nodules or hilum lymph nodes, presumably representative of old childhood-type tuberculosis, was noted in 12.5 per cent of the total subjects in the survey, in single posteroanterior films.

The figures in the preceding paragraphs furnish a rough idea of the incidence of tuberculosis in the area. The group studied formed about one-tenth of the population, but were not perfectly representative of the population at large. However, since the selection involved in the survey probably operated to exclude rather than include active tuberculosis (see the text), the figures 62 per thousand for case incidence and 27 per thousand for advanced disease are probably minimal figures.

The extent and density of the five instances of far-advanced and the eight instances of moderately advanced pulmonary tuberculosis discovered in the survey are shown in plate 1, and the extent and density of the 17 instances of minimal pulmonary tuberculosis in plate 2. Sanatorium cases are not included.

The majority of cases of advanced adult-type tuberculosis discovered appeared to be of chronic nature, conforming closely in X-ray appearance to the type of advanced disease usually seen in white patients.

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PNEUMOPERITONEUM IN INTESTINAL TUBERCULOSIS

Appraisal of the Various Therapeutic Procedures

DAVID SALKIN[1]

In the past few years great light has been thrown on the diagnosis and treatment of secondary ulcerative intestinal tuberculosis. Once looked upon as an inevitable forerunner of death, it is now regarded with greater hope, due to earlier methods of diagnosis by X-ray and definite and effective therapeutic aids. The older phases of treatment included attention to the original focus (the lung) by greater general and local rest and by the introduction of heliotherapy and vitamine therapy. The severer cases, however, continued to die; and it is in this group that artificial pneumoperitoneum has found its greatest usefulness. It has proved to be the greatest single means of relieving distressing symptoms, and, in conjunction with it, the more hopeful pulmonary cases have had some form of collapse therapy with resulting arrest of both pulmonary and intestinal disease. This form of air-therapy is still in its infancy and only recently has much attention been paid to it. We believe that its sporadic use has been due to the fact that its action has been surrounded with a certain "mysticism" and lack of properly controlled experiments.

HISTORY

The induction of pneumoperitoneum for therapeutic and diagnostic purposes dates back to 1895, when it was first used in cases of tuberculous ascites. Its use in ulcerative enteritis complicating pulmonary tuberculosis is more recent. Bainbridge (1) of New York reported in 1909 the use of intraäbdominal oxygen in 4 cases of tuberculous peritonitis associated with enteritis. Immediately following laparotomy, he inflated the abdomen with oxygen and claimed excellent results. In 1920 Rost (2) of the Indian Medical Service used oxygen inflations in 3 cases of tuberculous peritonitis, two of which were associated with ulcers in the bowel. All three cases achieved good symptomatic relief, and Rost attributed the results to a separation of the inflamed peritoneal surfaces

[1] Michigan State Sanatorium, Howell, Michigan, and Hopemont Sanitarium, Hopemont, West Virginia.

and the inhibition of the tuberculous process by oxygen. In 1921 Jelks (3) of Memphis followed up Rost's work and injected oxygen intraperitoneally in 3 cases of secondary ulcerative enteritis with great symptomatic relief. Laney (4) continued Jelks's work, and in 1924 reported the use of oxygen in 3 cases. The first case showed spectacular results but two later ones were failures. He concluded that this procedure "is not a curative but gives great relief." Hayes (5) reported its use in 6 cases in 1926. In four of the ulcerative group who exhibited ineffective results with diet, medication and intravenous calcium, the symptomatic relief was spectacular. But in 2 cases of the hypertrophic-stenotic type no relief was obtained. Hayes stated that it was the "most efficient means to relieve symptoms." In 1930 McCorkle (6) reported 9 cases which he had treated for five years with a total of 110 inflations with great symptomatic relief, but in each case a focus was previously removed by operation.

The more recent work has been stimulated by Banyai (7), who reported a series of 44 cases in 1931 and 100 cases in 1934 with symptomatic relief in 71 per cent and failures in 29 per cent. Klopstock and Schuller (8) in 1933 reported its use in 21 cases with marked relief of symptoms. Monaldi (9) reported 20 cases in 1933, obtaining good relief of symptoms in 70 per cent. Our work has been done following Banyai's 1931 report, and continued because of the successful use of pneumoperitoneum in the first few cases that had received no benefit from other modes of therapy.

TECHNICAL FACTORS

Pneumoperitoneum is best induced at a time about midway between meals so as not to interfere with the next meal or aggravate the sense of fulness following the previous one. Menstruation does not contraindicate its use. No previous preparation is necessary except that the bladder be emptied and undue flatulence be relieved by enema or pituitrin. If the patient is nervous a mild sedative may be given. The injection may be done anywhere along the anterior abdominal wall or by the subphrenic route: our routine site is through the rectus muscle about the level of the navel, thereby producing a muscular valve to prevent subcutaneous emphysema in patients with thin abdominal walls and considerable cough. An ordinary pneumothorax apparatus is used.

The skin is prepared with iodine and alcohol, and a fine needle is used to make a hypodermic injection of about 5 cc. of 0.5 per cent novocaine. A gauge-19 or -20 needle with a sharp oval bevel is attached to the pneumothorax machine and inserted along the anaesthetized

track with the injection of novocaine as the needle enters the peritoneal cavity. This is determined by a change in the sense of tissue resistance and a rapid flow of air. Initial pressures are useless. Avoid meteorism, proximity to scars and rapid distention. The initial dose varies from 400 to 800 cc., and refills consist of 500 cc. or more, depending upon the length of symptomatic relief and its relation to the amount injected. Refills are given only when symptoms return, which may vary from two to six weeks, and we have seen no benefit from repeated injections during an asymptomatic state. A sudden rise in pressure accompanied by a sharp pain signifies a lack of proper abdominal accommodation, due either to adhesions or a nonintraperitoneal inflation. As shown in a previous paper (10), accommodation is a highly efficient mechanism and a final pressure of about plus 8 cm. should not be exceeded. The entire process should be attended with a minimum of discomfort and be completed in 5 minutes. We use ordinary filtered air, which we have found to be at least as good as oxygen.

In over 1000 inductions we have had no deaths or shock, no known perforation of the gut, no formation of adhesions and no production of fluid. We have had several mild instances of subcutaneous emphysema and in three cases we have failed to enter the peritoneal cavity because of extensive adhesions as shown by previous laparotomy.

CLINICAL FEATURES OF AIR IN ABDOMEN

During an inflation the patient may feel a sense of tightness in the epigastrium followed by a feeling of fulness. At about 300 cc. of air there may be a dull ache or pain in one or both shoulders and in the back of the neck. If a phrenicotomy has been previously performed, no pain is felt on the same side. Further injection produces a sensation of increasing fulness but no pain. Following the inflation there is a period of peritoneal irritation for three or four days, characterized by sharp, shooting and fleeting pains in the abdomen, increased by exertion, and relieved by a snug abdominal binder and some codein and aspirin. This irritation may be mild, moderate or occasionally severe, and is of no symptomatic importance after the first inflation. The patient can tell the presence of air by a "rolling feeling" when he moves from side to side. No other postoperative care is necessary except when constipation becomes a marked feature; a small dose of mineral oil may then be given to initiate the process.

The objective features include the loss of liver dulness at about 300 cc. and the abdominal distention. The air gathers in the highest level, depending upon the posture, and the patient may be fluoroscoped by rolling him on one side. There is no method available to determine the amount of air present in the abdomen following inflation, but in the adult a total subphrenic air-space area of 10 to 12 cm. in the erect

position denotes at least 600 to 800 cc. of air. The diaphragm may show a greatly decreased range of activity and this fact has been used as a collapse measure in pulmonary tuberculosis, but we have never seen any definite collapse of the lung as a result of air in the abdomen. The effect upon the blood-pressure and blood-count is variable, and only slightly changed in most cases. A few cases do show a marked rise in systemic blood-pressure and an increase in the haemoglobin and erythrocytes of the peripheral blood.

USE OF PNEUMOPERITONEUM IN TUBERCULOSIS HOSPITALS

Far more important than the actual insertion of the needle into the abdominal cavity is the proper evaluation of symptoms referable to the digestive tract. Not all cases of dyspepsia in far-advanced pulmonary tuberculosis mean tuberculous involvement of the bowel. In fact, the nontuberculous dyspepsias outnumber the tuberculous in an institution for tuberculosis. At Michigan State Sanatorium during the year of 1933, two hundred cases of dyspepsia (other than simple anorexia) were studied. X-ray series were done on each case and only 90 (or 45 per cent) showed definite evidence of tuberculous enteritis. Since only the more severe dyspepsias were studied, we feel that, had all cases been included, the percentage would be much lower. It is our impression that in a tuberculosis sanatorium the nontuberculous dyspepsias are about four or five times more frequent than the tuberculous. A patient may have a definite enteritis and at the same time present symptoms resulting from cholecystitis, peptic ulcer, toxaemia, food dyscrasias, or overeating. Diarrhoea and pain do not mean intestinal tuberculosis any more than haemoptysis means pulmonary tuberculosis. All other conditions must be ruled out by a complete history, a knowledge of the course of various dyspepsias, X-ray and laboratory studies.

Several inflations are usually necessary to control symptoms. Pneumoperitoneum relieves the distressing symptoms wholly or partially but healing takes place by an increase of local and general resistance aided by the removal of the pulmonary focus of infection. In our series the maximum beneficial results were obtained by 37 per cent after one inflation, 38 per cent more after a second, 15 per cent more after a third and the entire group after four or five refills. Most of these cases required further inflation to maintain this state.

We have frequently seen accidents at the hands of careless operators, varying from puncture of the viscera to a stripping of the subperitoneal

space. As the procedure becomes more popular the accidents will increase, and it should therefore be performed only by those who are in a position to do follow-up studies.

DIAGNOSIS OF INTESTINAL TUBERCULOSIS

The best single means of diagnosing intestinal ulceration is the taking of a careful *history*. The presence of persistent or recurring symptomatology referable to the digestive tract in a case of pulmonary tuberculosis, especially when associated with a positive sputum and cavity-formation, should point to a thorough investigation. If such a case is negative roentgenologically (and is not associated with phrenicotomy, pleural effusion or secondary disease like cholecystitis), then it should be considered as one of tuberculous enteritis until proved otherwise. One must keep in mind that severe pain and diarrhoea are seen in cases which at autopsy show no organic lesion and must be regarded as due to tuberculotoxaemia.

Proved cases of tuberculous enteritis may be entirely asymptomatic. Crawford and Sawyer (11) observed this in 3 per cent of their cases and in a series of 80 autopsies we have observed it in 8 cases (or 10 per cent). The symptoms may be mild, with an extensive lesion, or severe, with a small lesion, and may simulate any abdominal and many general diseases. In a series of 155 cases the chief symptoms occurred in this order of frequency: anorexia 85 per cent, crampy pain 78 per cent, nausea 68 per cent, diarrhoea 64 per cent, flatulence 50 per cent, vomiting 45 per cent, epigastric distress 30 per cent. These are followed by constipation, tenderness in right lower quadrant, pyrosis, acid regurgitation, constipation alternating with diarrhoea, gross blood in stools, attacks of appendicitis and allergic disturbances. Of these cases, 4 per cent showed only gastric symptoms and 5 per cent had stools of normal consistency, color, and odor with no undigested food. In 2 cases we have seen symptoms of "peritonismus" characterized by a rigid tender abdomen, constipation, mild fever and leucocytosis of five to seven days' duration and disappearing spontaneously. At autopsy both cases showed enlarged mesenteric lymph nodes, ulcerated bowel and a normal peritoneum.

The *X-ray method* of Brown and Sampson (12) is the best positive means of diagnosis. We have found both the oral and enema examinations essential, with the oral route being about seven times more diagnostic than the enema. The enema is rarely positive when the oral examination is negative. Brown and Sampson claim a high degree of

PLATE 1

Fig. 1A. Case presenting abdominal cramps and epigastric distress. X-ray examination shows a suspicious defect only at the 12-hour oral examination.

Fig. 1B. Same case 10 days after one air inflation of 600 cc. Disappearance of symptoms and normal X-ray appearance at all hours.

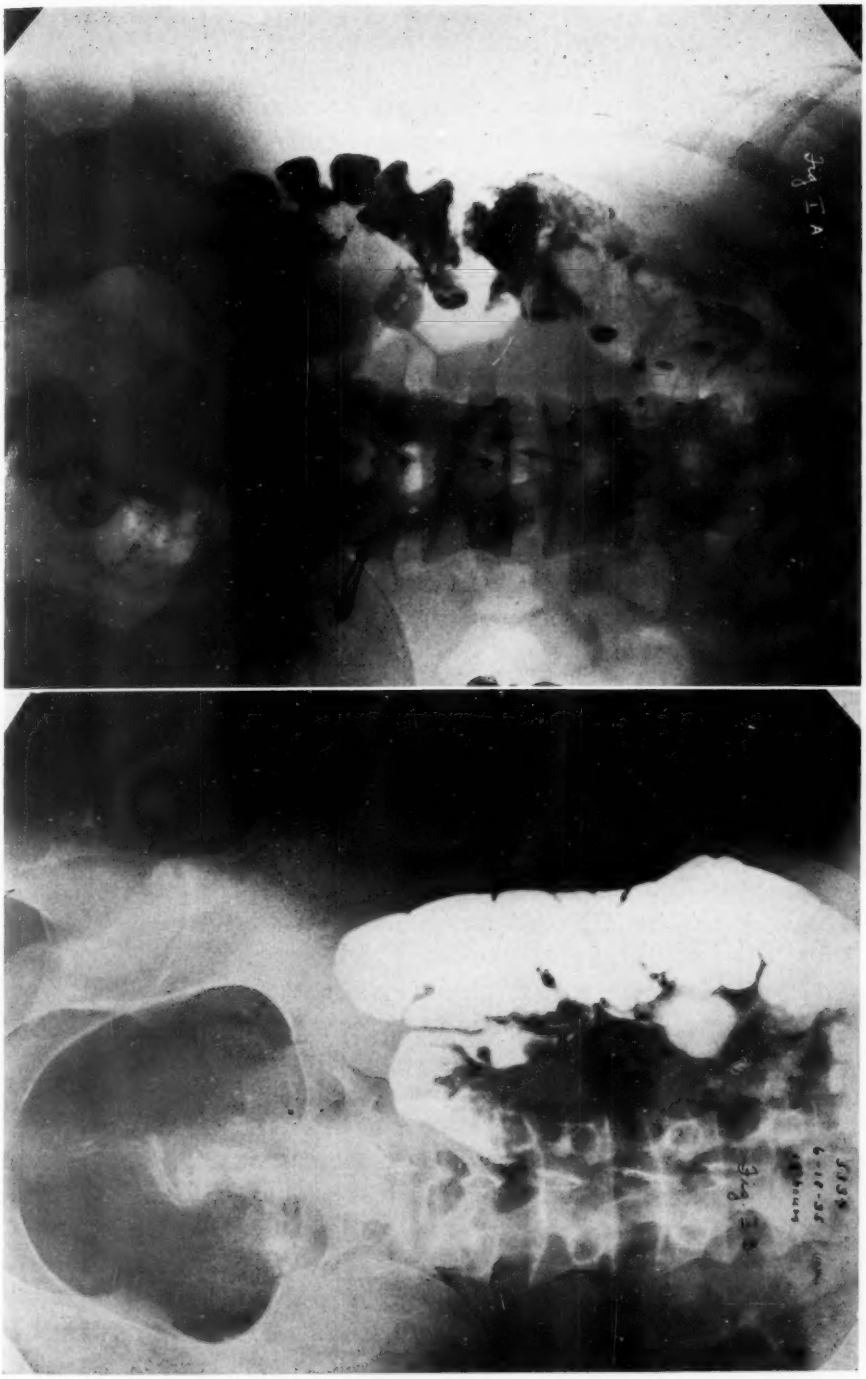

Fig. 1A

Fig. 1B

PLATE 1

accuracy for the ray while Steinbach (13) believes it to be only 50 per cent correct. We are inclined to agree with both authors, particularly with Steinbach, whose own figures show that a positive X-ray diagnosis is 90 per cent correct and a negative one only 30 per cent. Our results show that a positive X-ray diagnosis is correct in over 95 per cent, a negative one in 30 per cent, and a suspicious one after two examinations in less than 50 per cent. Thus a negative report in the face of suspicious symptoms should be regarded as only 30 per cent reliable.

We wish to introduce *pneumoperitoneum* as a diagnostic agent in intestinal tuberculosis. In cases with negative or suspicious X-ray findings, one or two inflations will produce a striking clinical change in symptomatology, and subsequent X-ray examinations may show a positive result or some other change such as decrease in local spasm or motility. (See figure 1.) We have used pneumoperitoneum on the cases of 12 perfectly normal digestive tracts, of which not one showed any change in the gastrointestinal X-ray series. Several suspicious cases of intestinal tuberculosis cleared up, and showed definite X-ray changes after one inflation. One case, diagnosed as enteritis and to which air was given, showed no signs of relief, and at autopsy presented a retrocaecal subacute appendicitis with no evidence of enteritis. We have obtained marked relief with air inflation in several cases of salpingitis in which extensive pulmonary disease contraindicated laparotomy; of these, only one was of tuberculous origin. No results were obtained with air inflation in cases of neurosis and gall-bladder colic.

PRESENT TREATMENT OF INTESTINAL TUBERCULOSIS

The chief modes of therapy used in intestinal tuberculosis at the present time are diet, medication, calcium, surgery, heliotherapy and vitamine therapy.

The *diet* in general use is bland and has "no roughage, vegetables, raw fruits, cellulose-rich foods, spices or soups; and meals must be of small bulk." The lack of variety in such a diet, its monotony, and the fact that we have very rarely seen it produce any definite benefit has led us to discard it. We now prescribe a *selective* or elimination diet in which only those foods which are known to aggravate symptoms are omitted, and the patient is allowed such foods as spices, soups, and vegetables, provided they do not aggravate the symptoms. As a general rule, such a diet is preferred by the patient and we have not observed any detrimental effects. This selection is purely individual and varies with each

patient. Many irritating foods may be well tolerated after changing the intestinal gradient with pneumoperitoneum, and 4 patients allergic to milk and strawberries lost their sensitivity shortly after air inflation. It appears that many foods which aggravate the symptoms do so by allergic phenomena rather than by mechanical irritation, and this allergy is itself greatly influenced by the character of gastrointestinal motility.

Almost all known *drugs* have been used at some time. We have found the following to be generally useless: castor oil; parathyroid extract; such antiseptics as salol, dimol, beta-naphthol, hexyl-resorcinol; such astringents as lead, iron, tannates and gallates; enemata and lavages. We have often seen great temporary benefit with belladonna, opiates, bismuth, calcium and barium. Mild cases may be completely controlled with these drugs until spontaneous remission or cure results. We have used such cases as our controls in evaluating the other modes of therapy. In a control series of 28 cases, McConkey found improvement in only one (or 3.5), whereas, we have seen it in 11 out of 90 cases (or 12 per cent).

Calcium Therapy

In 1924 Roberts (14) reported a series of 70 cases of intestinal tuberculosis treated with intravenous calcium chloride. He claimed 85 per cent beneficial results and 15 per cent failures. Our own experiences have been rather disappointing. Using calcium both intravenously and intramuscularly we have seen benefit in only about 15 per cent, which is little better than the control series. It is true that we have used it only in the more severe cases but, even when used with other modes of therapy, the general results have not been encouraging for the control of either pain or diarrhoea.

Surgery

Surgery has its place usually in cases of stenosis or perforation. Unfortunately, both these complications occur in cases with far-advanced pulmonary disease in which operative intervention would only hasten the end. The best surgical results occur in the more hopeful pulmonary cases having stenosis, disease localized to the appendix and the hypertrophic form. Frequently a simple opening and closing of the abdomen (with or without appendicectomy) results in symptomatic relief for varying periods of time (15). However, these cases often return later with severe progressive intestinal disease. It is possible that the good results of laparotomy are due in part to the introduction of air into the abdomen.

Operation should be done by one familiar with the pathology of the disease and with the coöperation of the roentgenologist. Not infrequently we have seen at autopsy shallow ulcers with no gross reaction in the serosa, whereas X-ray examination revealed the extent of the pathological process fairly well. In one case of failure to respond to all modes of therapy including pneumoperitoneum, simple laparotomy showed extensive fibrotic bands in the small gut; no further surgery was done, yet the patient was greatly relieved of his distressing symptoms. In this disease, surgery has a definite but very limited rôle.

Heliotherapy

Brown and Sampson reported 85 per cent good results with the use of ultraviolet light. McConkey reported 60 per cent good results in a series of 50 cases. In our own series the results were not nearly as good and the time for improvement was prolonged. In both Michigan State Sanatorium and Herman Kiefer Hospital the heliotherapy results are so entwined with natural remissions and pulmonary-collapse therapy that it is impossible to evaluate the method properly. Excluding these factors, we have particularly observed 20 cases which had light for at least four months and up to 3 years. In this group 3 cases showed complete symptomatic relief, two showed partial relief, and fifteen were failures resulting in 25 per cent benefit and 75 per cent failure. Although this series is small we feel that it portrays our general impression in a much larger number of cases. We have found no relation between the results of the lamp and the extent of the intestinal lesion. We do feel that there is a definite relationship to the severity of the symptoms, those having moderate symptomatology resulting in 50 per cent relief and those with severe symptoms in 15 per cent. These results are inferior to those of vitamine therapy. Occasionally a case is seen which is helped by the lamp, but not by vitamine therapy, but this is uncommon. We believe that heliotherapy is time-consuming, expensive and does not deserve the popularity given it.

Vitamine Therapy

The use of a high-vitamine diet in intestinal tuberculosis was introduced by McConkey (16), and we believe that it is a distinct advance in the therapy of this disease. We have used liberal doses of codliver oil, haliver oil, viosterol, tomato juice and orange juice. The routine followed is to give, twice daily, one ounce of codliver oil floated upon three

ounces of tomato juice served cold; when the codliver oil has tended to produce nausea, haliver oil has been substituted with equally good results.

Steinbach (17) has stated that vitamine therapy is of no benefit and is not curative. McConkey claims benefit in 68 per cent and failure in 32 per cent of cases. In a carefully studied series of 60 cases receiving vitamine therapy, from a period of four months to three years or more, we have found improvement in 50 per cent and failure in 50 per cent. These figures are not final in themselves, but require closer analysis. The results bear no relation to the extent of the intestinal lesion, but they do have a definite relationship to the degree of symptomatic severity. Our series comprises 3 cases with mild, 20 with moderate and 27 with severe symptoms. Of the mild cases, one proved to be a failure and the group is too small to be taken into statistical study. Of the moderate cases, 67 per cent showed improvement, and of the severe cases, 25 per cent. We feel that such a division is necessary, for one worker may be dealing with severe cases only and another with moderate cases only and both reach conflicting conclusions. The method of classification will be discussed later.

Pneumoperitoneum Therapy

In the series treated with pneumoperitoneum certain control features were instituted. Almost every case had some previous form of therapy, which produced a failure or partial result. With the beginning of air therapy the patient was placed on the ordinary hospital diet. No food of any nature was withheld, and the menu included raw fruits and vegetables, corned beef, fish, soups, pork, fried foods, sugars and spices. All medication was discontinued and given only to subsequent failures or partial results. Heliotherapy, vitamine therapy and calcium were also discontinued.

This series comprises 95 cases, including 76 whites (39 males and 37 females), 17 Negroes (2 males and 15 females) and 2 male Indians. Pneumoperitoneum was first begun by us in November, 1931; and the cases mentioned were studied at Herman Keifer Hospital, Detroit, Michigan; Michigan State Sanatorium, Howell, Michigan; and Hopemont Sanitarium, Hopemont, West Virginia.

Previous therapy included 68 cases on bland diet with only definite though partial relief in 4 cases. Medication was given to 87 cases, with results varying from partial temporary benefit to failure. Vitamine therapy was given to 48 cases for at least three months with partial

results in 4 and failure in 44 cases. Ultraviolet light was given to 20 cases with partial results in 3 and failure in 17 cases. Seven cases had intravenous calcium with partial results in 1 case.

In the past two years we have followed the routine of giving air therapy only to those cases that have had poor or no results with medication, vitamine or light therapy. Almost every case (including most of the failures) showed some physiological change from within a few minutes to a few days after inflation and before any change could occur in the lung. One case of a partially good result is not included because the patient began subsequently to complain of pain which was relieved by simple paracentesis. The neurogenic factor will be discussed later.

The results obtained with air therapy show good or complete symptomatic relief in 73 per cent, good partial relief in 9 per cent, and failure or poor partial relief in 18 per cent. (See table 1.)

TABLE 1

End-results of pneumoperitoneum therapy

SYMPTOMATIC RESULT	WHITE RACE: 76 CASES	NEGRO RACE: 17 CASES	TOTAL IN SERIES: 95 CASES
	per cent	*per cent*	*per cent*
Good or complete	72	76	73
Good partial	12	—	9
Failure and poor partial	16	24	18
Some improvement	84	76	83
No improvement	16	24	17

Before comparing the results of the various modes of therapy we shall first discuss the basis of classification into degree of symptomatology and extent of the lesion.

CLASSIFICATION OF EXTENT OF LESION

We realize the difficulty underlying an attempt to classify the extent of the lesion by the X-ray, for no two workers would probably agree as to the exact extent of the lesion, and it is a known fact that X-ray and autopsy may not correspond. Nevertheless, in the great majority of cases an approximate extent of the spastic deformity is fairly well determined and it corresponds well with autopsy findings.

The classification is as follows: A Class 1 lesion is one involving the small gut, caecum or a localized area about the ileocaecal valve or caecum and appendix. A Class 2 lesion is one which extends into the

ascending colon but not beyond the hepatic flexure. A Class 3 lesion is one which extends beyond the hepatic flexure.

We realize that such a scheme is certain to provoke criticism, and we shall therefore discuss its advantages and disadvantages. In the first place, the classification does not take the small gut as of great importance. However, tuberculosis of the small intestine alone is very rare and usually extremely difficult to diagnose by X-ray. Its symptomatology is very indefinite, and it is usually involved with the large bowel because the common symptoms of pain and diarrhoea are usually referable to the large intestine and terminal ileum. Other symptoms, such as anorexia, vomiting and flatulence, are most commonly caused by a disturbed motor gradient in the large gut. Actually, the omission of the small bowel from such a scheme does not affect it appreciably.

Another possible disadvantage of such a scheme is that a small or localized ulceration may produce a widespread or generalized deformity roentgenologically. However, even when the actual lesion is small (as shown by laparotomy or autopsy) and the X-ray defect is widespread we consider the lesion by its roentgenological extent because the physiological effect is equivalent to ulceration of a large portion of the gut. Again, where autopsy shows an involvement of the entire colon and the X-ray shows only a Class-2 lesion the case is again regarded by its roentgenological extent. An ordinary spastic colon may show no organic lesion yet it is productive of a widespread functional disturbance. Whatever the actual extent of the lesion, only the extent of the physiological derangement is used as the basis and this is best determined by X-ray.

How does the X-ray method of determining the extent of the lesion correspond with actual autopsy findings? We have used the enema frequently in conjunction with the meal, but we have found the oral method of Brown and Sampson more efficient in determining the extent of physiological disturbance. A comparative study of 16 cases was done. All these cases had X-ray studies shortly before death and the results compared with actual findings. Of this series 12 cases came to autopsy, one case had a laparotomy and 3 cases had proctoscopic examination. It was noted that 11 out of the 15 cases compared favorably with both X-ray and anatomical findings; 3 cases showed more disease anatomically and 2 cases showed less disease. The differences however were not great.

CLASSIFICATION OF DEGREE OF SYMPTOMATOLOGY

Whether a patient with intestinal tuberculosis complains of only one symptom or ten the importance does not lie in the number but in the

severity of complaints. The symptoms may be only mild and slightly distressing and these cases are highly amenable to medication therapy. Moderate symptoms comprise a greater degree of distress, and the patient may complain of nausea, occasional vomiting, moderate degree of crampy pain and one or two loose bowel movements daily. Severe symptoms include frequent vomiting, marked flatulence, severe diarrhoea and crampy pain requiring narcotics. Cases are grouped readily as mild or severe, and the intermediate ones are classified as moderate. We have been accustomed to divide the symptoms as gastric or intestinal. The gastric group includes pyrosis, anorexia, upper abdominal distention, epigastric pain, nausea and vomiting. The intestinal group includes lower abdominal distention, crampy pain and diarrhoea. Alvarez (18) has shown that a lesion in the caecum may produce gastric symptoms, depending upon the degree of upset of the intestinal gradient or the intensity of the stimulus. Intense stimulation from a small lesion may cause a greater symptomatic disturbance than mild stimulation from an extensive lesion. It is a common observation that a small area of ulceration may result in severe pain, diarrhoea and vomiting, whereas many extensive lesions are seen at autopsy, which produced no symptoms at all during life and whose presence was unsuspected. We hope to show later that the degree of intestinal disturbance depends upon the number and intensity of the stimuli produced, which in turn depend upon the extent of the lesion and local neuromuscular irritability. Thus a given case of intestinal tuberculosis may be Class 3 with mild symptoms or Class 1 with severe symptoms.

CLASSIFICATION OF PNEUMOPERITONEUM RESULTS

The degree of symptomatic relief obtained by air therapy depends greatly upon the extent of the lesion. (See table 2.)

TABLE 2

Pneumoperitoneum results based upon extent of lesion

CLASS	NUMBER OF CASES	GOOD OR COMPLETE	PARTIAL	FAILURES
		per cent	*per cent*	*per cent*
1	9	89	11	—
2	31	77	13	10
3	39	61	9	30

The greater the extent of the lesion the larger is the percentage of failures. In the Class-1 and Class-2 cases symptomatic relief is the rule,

whereas in advanced cases there are 30 per cent of failures. The results also bear a definite relationship to the severity of the symptoms. All of the moderate cases and 70 per cent of the severe ones showed good symptomatic relief. (See table 3.)

TABLE 3

Pneumoperitoneum results based upon severity of symptoms

SYMPTOMS	NUMBER OF CASES	GOOD OR COMPLETE	PARTIAL	FAILURES
		per cent	*per cent*	*per cent*
Mild	—	—	—	—
Moderate	7	100	—	—
Severe	88	70	10	20

COMPARISON OF THE VARIOUS THERAPEUTIC PROCEDURES

Comparative evaluation will be made between the three best modes of therapy, namely, ultraviolet light, vitamine therapy and pneumoperitoneum. With a more detailed classification as outlined, a better estimation can be made of the value of each procedure in a given group of cases. Table 4 shows the number and types of cases studied in each group.

TABLE 4

Types of cases studied in each series

	HELIOTHERAPY		VITAMINE THERAPY		PNEUMOPERITONEUM	
	Number	Per cent	Number	Per cent	Number	Per cent
Extent of lesion						
Class 1	2	10	5	8	9	9
Class 2	8	40	34	56	28	29
Class 3	10	50	17	28	40	42
Unknown	—	—	4	8	18	20
Symptomatic severity						
Mild	—	—	3	5	—	—
Moderate	5	25	30	50	7	8
Severe	15	75	27	45	87	92

In this series the great majority of the heliotherapy and pneumoperitoneum cases fall into the Class-3 and severe symptomatology, whereas most of the vitamine cases fall into the Class-2 and moderate symptomatology.

That the results obtained with light and vitamine therapy do not depend upon the extent of the lesion is shown in table 5.

TABLE 5

Relationship of extent of lesion to vitamine and light therapy

THERAPY	EXTENT OF LESION	NUMBER OF CASES	SYMPTOMATIC RESULTS		
			Good	Partial	Failure
			per cent	*per cent*	*per cent*
Vitamine	Class 1	5	—	20	80
	Class 2	34	24	18	58
	Class 3	17	24	36	40
Light	Class 1	2	—	—	100
	Class 2	8	13	—	87
	Class 3	10	20	—	80

Since pneumoperitoneum is proportional to both symptomatic severity and extent of lesion, and the results of light and vitamine therapy are not proportional to the extent of the lesion, then the three modes of therapy will be compared from the standpoint of degree of symptomatology. (See table 6.)

TABLE 6

Comparison of vitamine, light and air therapy from the standpoint of symptomatic severity

SYMPTOMS	RESULTS	
	Good or Partial	Failure
Mild	? Air 67 per cent Vitamine ? Light	? Air 33 per cent Vitamine ? Light
	per cent	*per cent*
Moderate	100 Air 67 Vitamine 40 Light	— Air 33 Vitamine 60 Light
Severe	80 Air 27 Vitamine 15 Light	20 Air 73 Vitamine 85 Light

In the mild symptomatic group vitamine therapy gives relief in two-thirds of the cases, but, since this group comprises only 3 cases it cannot be taken into a statistical study. No mild cases are present in the light and air groups. In the moderate symptomatic cases air gave relief to

the entire seven, vitamines gave relief in 67 per cent, and light in 40 per cent. In the severe symptomatic group air gave relief in 80 per cent, vitamines in about 25 per cent, and light even less. Both light and vitamines act best in the cases with moderate symptoms, whereas air is particularly beneficial in the severe cases. The general order of excellence in relieving symptoms is air, then vitamines and, finally, ultraviolet light.

STUDY OF THE FAILURES OBTAINED BY THE VARIOUS METHODS

The failures of *ultraviolet light* were subjected to further therapy. In a series of 20 cases, including 18 failures and 2 partial results, vitamine therapy was further added. The two partial results showed a slight increase in symptomatic relief but the end-result was not good; air therapy was added with good results. Of the 18 failures, vitamine therapy was added in 13 cases with good relief in one case and failure in twelve. Ten of these 12 cases had further air therapy, with good results in 8, partial results in one case, and failure in one. Five cases received air without previous addition of vitamines, with good results in 3 cases and failure in 2 cases. Addition of medication to the ultraviolet light cases showed no appreciable change in the end-results.

Summarizing: In the above series of 20 cases the addition of vitamine therapy to 13 cases resulted in 12 failures and one good result; and the addition of pneumoperitoneum to 17 cases gave 13 good results, one partial and 3 failures.

The *vitamine group* includes 72 cases in which a partial result or a failure was obtained. Good partial result was obtained in 15 cases, all of which had additional palliative medication, with good results in 12 cases and failure in 3 cases. Of these 12 cases, 7 showed definite improvement coincident with improvement in the lung and medication helped to tide over a critical period. The remaining 3 cases had pneumoperitoneum with good results in all. Poor partial results were obtained in 4 cases. Two of these had additional ultraviolet light, which still increased the benefit to some extent but not fully; and the addition of air to these produced good results in both. One case had additional medication with fair result until the time of death. The other case with medication improved as the lung lesion showed regression of activity. The failures comprised a group of 53 cases all of which had additional medication. Seven cases improved coincidentally with the pulmonary lesion, one case showed a spontaneous improvement, and the 3 cases were failures.

Pneumoperitoneum was given to 42 cases with complete results in 31, partial in 5 and failure in 6 cases.

Summarizing: One may say that the addition of medication in the above 72 cases gave 5 good results, 9 partials and 58 failures. Fifteen of these cases (or 21 per cent) were favorably influenced by regression of the pulmonary lesion. The addition of ultraviolet light to two cases resulted in additional symptomatic relief in both. The addition of pneumoperitoneum to 42 cases gave good results in 31, partial in 5 and failure in 6 cases.

FAILURES OF PNEUMOPERITONEUM THERAPY

This group of 24 cases includes 8 cases which had shown good partial results, and 16 poor partials and failures. The 8 good partial cases did well when therapy was supplemented with medication; in 2 cases calcium had beneficial results. Of the 16 remaining cases, 12 died as a direct result of the intestinal tuberculosis. Of these, 5 had vitamine therapy with medication, which resulted in failure; 5 more had only medication with failure; 2 cases had additional calcium therapy with failure; 2 cases had additional light therapy with failure. Four cases are still alive. One case (S. V.) did not respond to light, medication and air but improved spontaneously. One case (E. H.) did not respond to light, vitamines and air and again improved spontaneously. One case (S. C.) had light, vitamine, air, medication, calcium and an arrested pulmonary lesion, with failure, but improved spontaneously at a later date. One case (F. P.) had all these modes of therapy with failure, but improved after a simple exploratory laparotomy. None of these 4 cases bore any relationship to the condition of the lung.

Summarizing: One may say that, when pneumoperitoneum fails, vitamine, light and calcium will probably also fail, and most of these cases will probably die of their intestinal tuberculosis. It is a noteworthy fact that where drugs, such as belladonna or laxatives, are used in conjunction with pneumoperitoneum their effects are striking with small doses.

RELATIONSHIP OF THE PULMONARY LESION TO INTESTINAL TUBERCULOSIS

Since intestinal tuberculosis is a complication of the pulmonary form one may expect a close connection between the two conditions, and this is true in a large number of cases. Most cases of intestinal tuberculosis

with mild symptoms and some with moderate symptoms will improve as the lung improves. It is very difficult to present figures on such a problem, but we believe that probably 20 per cent or more of all cases of intestinal tuberculosis will improve without any therapy other than healing of the pulmonary lesion, and with collapse even more quickly. Together with medication, pulmonary collapse would probably affect beneficially at least from 35 to 40 per cent of all secondary intestinal tuberculosis. Severe cases need other therapy. Whatever the procedure used (vitamine, light, air) its results are further increased by improvement of the pulmonary lesion. This fact is not taken into serious consideration by many authors.

However, many cases of intestinal tuberculosis run a course of their own, unaffected by the pulmonary lesion and occasionally aggravated by collapse therapy. We have known some cases whose symptoms were initiated by phrenicotomy, especially left; thoracoplasty; a pneumothorax effusion, which accompanied an increased pulmonary collapse with negative sputum; or, rarely, a pneumothorax. Our worst case of intestinal tuberculosis occurred 3 months after a successful thoracoplasty with negative sputum; all modes of therapy failed and the patient died as a result of intestinal symptoms. With the addition of air therapy in intestinal tuberculosis such cases can be more easily controlled and should not contraindicate any form of collapse therapy. In fact, the presence of intestinal tuberculosis is a strong indication for collapse therapy if the pulmonary lesion is not too extensive.

THE NEUROGENIC FACTOR IN AIR THERAPY

Occasionally a case is encountered in which the administration of air intraperitoneally results in such good relief of symptoms that the patient may begin to complain of symptoms in order to get air, and, in these, a mere insertion of the needle without introduction of air may give relief. Such cases have had definite relief formerly and have begun to depend upon air as a safeguard against the return of symptoms. The same phenomenon occurs in pneumothorax, in which a patient may ask for a refill simply because he is nearing the end of his interval and wants to prevent or "get the jump" on anything that is about to happen. The psychogenic phase of therapy is important in the use of any procedure. The changes in the radiological aspect of the gastrointestinal tract and repeated beneficial effects of pneumoperitoneum therapy relegates the psychic phase of therapeutic relief to a minor rôle.

SYMPTOMATIC RESULTS OF AIR THERAPY

The results obtained are best explained by Alvarez's theory of intestinal gradient. Since most ulcerations occur about the ileocaecal region, disturbances in gradient affecting the more distant portions of the gut will be first benefited even by a small reduction of local excitation. Probably for this reason the symptoms of pyrosis, nausea and vomiting, which are associated with gastric motor changes are first affected. These symptoms are not only most frequently benefited, but often are the first to disappear following air induction and may not return.

Pyrosis and *acid regurgitation* are symptoms which are associated with motor changes in the lower oesophagus and upper portion of the stomach

TABLE 7

Shows the detailed results of air therapy upon the individual symptoms

SYMPTOM	NUMBER OF CASES	SYMPTOMATIC RELIEF		
		Good	Partial	Failure
		per cent	*per cent*	*per cent*
Pyrosis	9	100	—	—
Acid regurgitation	12	100	—	—
Epigastric distress	24	96	—	4
Vomiting	44	93	—	7
Nausea	67	92	4	4
Flatulence	50	90	4	6
Anorexia	82	84	—	16
Constipation	17	80	—	20
Crampy pain	80	80	3	17
Tender right lower quadrant	24	75	20	5
Diarrhoea	64	72	8	20

and in this series have always been relieved by air therapy. One must remember that only pyrosis produced by intestinal tuberculosis is thus affected; we have not tried it on functional or allergic pyrosis. The *epigastric distress* or pain may simulate closely that of peptic ulcer and may be immediate or delayed after meals. Its production is also associated with the gastric motor function and frequently shows immediate and spectacular disappearance after air therapy.

The disturbance of gradient in the stomach produced by a localized lesion in the caecum may manifest itself in three ways; there may be excessive gastric motility and tonicity with a rapid emptying time; there may be a stasis in the cardia with spasm of the body and pyloric area for more than 6 hours; or in certain cases the stomach may be dilated

and atonic. Such changes in tone and motility are intimately associated with the production of nausea and vomiting, and correction of the disturbed gradient quickly changes the X-ray picture, giving it a more normal appearance and producing relief of nausea and vomiting.

Flatulence in the upper abdomen may produce severe disturbances by embarrassing respiration in a patient whose vital capacity is low. We hope to show in another paper the serious effects gaseous distention of the small gut may have upon the respiration of the diseased lung. The dyspnoea a patient may have immediately after a meal or with gaseous distention is well known. When the upper abdominal distention aggravates or causes dyspnoea in a patient, one should give air therapy, for relief is often immediate. One must remember, however, the increased technical dangers in cases of meteorism.

Anorexia in cases of intestinal tuberculosis is due to general toxaemia and disturbances in motor gradient. The fact that 84 per cent of the cases had a definite increase in appetite shows the importance of the latter factor. Good appetite may result in patients with high fever, toxaemia and a hopeless lung lesion. Occasionally a state of bulimia results and one may crave for certain types of foods. Cases in which anorexia is not relieved are usually those with a high fever and generalized toxaemia or a bilateral pneumothorax.

Crampy abdominal pain may be relieved without relief of diarrhoea. We consider the relief of pain to be one of the greatest clinical values of pneumoperitoneum. When relief is partial, belladonna is a useful adjunct. Often the relief occurs on the operating table during the inflation. A common *triad* of symptoms encountered comprises constipation, crampy pain and pain in the right lower quadrant. In this type of case, air therapy produces striking results with the constipation; in fact, the addition of small doses of laxatives often results in diarrhoea. Tenderness in the right lower quadrant is very frequently benefited, and some of the most unusual results belong in this group. Several cases had typical recurring attacks of appendicitis with pain in right lower quadrant, tenderness, slight rigidity and leucocytosis. X-ray showed a tuberculous enteritis with appendicitis as part of the picture; and air therapy gave rapid relief. One such case with a white blood count of 19,000 had immediate relief of pain and the blood count taken next day showed 11,000 cells.

Diarrhoea is the most difficult of the symptoms to treat. Correction of this symptom should mean a normal stool about once a day and pro-

longed contact of the food with the colon. Some of the cases had only one watery or soft stool daily, but the ray showed that in 8 hours the entire meal was in the rectal ampulla and stayed there for another 10 to 16 hours. Following successful air therapy the stools are decreased in number, become normal in consistency, color and odor, and contain no undigested food. We have not noted any special effect of the air therapy upon *intestinal haemorrhage.* Even in successfully treated cases which came to autopsy eventually, many haemorrhagic ulcers were noted, in which only slight irritation may cause bleeding. Apparently change in muscular spasm about these ulcers has no effect upon the tendency of exposed vessels to bleed. In fact the possibility to bleed is greater on account of the prolonged contact of the food with the ulcerated area but actually we have not seen it.

We have seen changes in *food idiosyncracies* following air therapy. Cases who previously would have nausea, vomiting or distress after eating such foods as raw milk or fruits would tolerate these articles without difficulty after pneumoperitoneum. They would often recur with other symptoms and again be relieved by air. Patients frequently remark upon their ability to eat bananas or eggs without distress or medication after air therapy. Two noteworthy cases had typical hives after eating strawberries, other fruits and raw milk; after air inflation they would be able to ingest these foods without production of allergic signs until other symptoms recurred. With every air inflation the nonallergic state would appear, until after 6 refills the hives did not return. We hope to show that air therapy produces changes in the intestinal motor gradient, and that these changes affect such allergic states.

THEORIES OF ACTION OF PNEUMOPERITONEUM

The various theories of action of pneumoperitoneum are given below and the author's name is in parentheses.

Mechanical:

1: Increased intraäbdominal pressure. (Laney, Monaldi.)
2: Serosal irritation by oxygen. (Laney.)
3: Separation of inflamed surfaces. (Rost.)
4: Displacement of abdominal viscera. (Monaldi.)

Vascular:

5: Relief of intestinal capillary stasis. (Rost.)
6: Secondary hyperaemia produced by oxygen. (Banyai.)

Oxidation:

7: Increased cellular activity by direct oxygenation. (Jelks.)

8: Supersaturation of body and blood-stream with oxygen. (Jelks, Bainbridge.)

Biological:

9: Inhibitory effect of oxygen upon pathologic processes. (Rost.)

10: Stimulation of antibodies by endothelial excitation. (Jelks.)

Comments

We have previously shown that it is very difficult to increase intraäbdominal pressure, and we have failed to observe any effect of air peritoneally upon a normal bowel. Most cases have relief of symptoms long after the air has been absorbed, thus ruling out changes in intraäbdominal pressure. Displacement of abdominal viscera occur frequently after pulmonary-collapse therapy with no effect upon the intestinal symptoms. All theories based upon the action of oxygen must be modified, since air and pure nitrogen have given us equally good results. In fact, the peritoneal irritation is milder and of shorter duration with nitrogen than with air or oxygen. We have failed to find any detrimental effects of oxygen upon a tuberculous process, and it has been shown experimentally that oxygen intraperitoneally has a negligible effect in raising the oxygen content of blood. In successfully treated cases, later observed by laparotomy or at autopsy, there were no evidences of either increased local vascularity or relief of capillary stasis any more than found ordinarily in untreated cases. Any change in the blood-field is secondary and negligible. The separation of inflamed surfaces cannot be taken into serious consideration because relief is often obtained after the air has been absorbed and the surfaces are again in contact, and relief has also been seen in cases of shallow ulceration with no serosal reaction. We have not seen any greater degree of fibrosis or healing with air therapy than ordinarily seen.

PATHOLOGICAL PHYSIOLOGY OF INTESTINAL TUBERCULOSIS

The physiology of the ulcerated bowel in intestinal tuberculosis is of great interest but in almost entire darkness. It is ordinarily stated that the localized inflammatory process produces an increase in the tone or spasm, which may further be increased by involvement of the intrinsic nerve plexuses. It is believed that the number and location of the ulcers play an important part in the production of the diarrhoea, and that the

crampy pain is caused by increase in muscle tonus especially at the site of ulceration.

The stomach and intestine are independent organs and have an intrinsic nerve-supply from the plexuses of Auerbach (myenteric) and Meissner (submucosal), which enable them to contract and dilate locally and generally. The extrinsic nerves regulate still further the action of the gut; parasympathetic stimulation produces contraction and increased tonus, whereas sympathetic stimulation causes relaxation and an inhibition of tonus. The importance of the action of the extrinsic nerves is best shown by the part the sympathetics play in some cases of Hirschprung's disease (19). The parasympathetic nerves also contain secretory elements. Important nerve-fibres frequently overlooked are those concerned with afferent impulses. They originate from cells in the cerebrospinal system and travel mainly in the same sheaths with sympathetic fibres. Although the intestinal mucosa is not richly supplied with afferent fibres, yet these play an important rôle in the production of symptoms. Usually, the impulses they mediate remain at a subconscious level. In cases associated with abnormal tonus and peristalsis, these impulses are interpreted as pain, nausea, distress or pyrosis.

From our experimental work still in progress we have begun to regard the production of spasm and irritability occurring in the ulcerated bowel as due to two factors:

1: Localized reaction with resulting spasm due to the direct action of toxins upon the exposed muscle and nerve-endings. (Intrinsic factor.)
2: An afferent-parasympathetic efferent reflex from the irritated afferent nerve endings to the central nervous system and return *via* the parasympathetics to the site of ulceration and, in many cases, the adjacent segments. (Extrinsic factor.)

The ulcerated segment is often seen on an X-ray plate filled with gas and presenting a normal appearance, but, as soon as food or barium is given, a state of spasm results, which ejects the irritant from the ulcerated area. This spasm may then be regarded as a protective reflex of the visceromotor type. No doubt both intrinsic and extrinsic factors play a part in the production of symptoms; we believe the main factor is extrinsic in about 80 per cent of all cases.

Any theory presented regarding the pathological physiology of the ulcerated bowel must explain the following facts:

1: A small lesion may be present with severe symptoms and an extensive one with few symptoms
2: Ulceration may be present without any symptoms
(a) With X-ray changes
(b) Without X-ray changes
3: Symptomatic states may alternate with asymptomatic for varying periods of time
4: Antispasmodic drugs have little or no action
5: Pneumoperitoneum may duplicate all the above features with recurring symptomatology and X-ray changes

KNOWN FACTS CONCERNING PNEUMOPERITONEUM

Prior to discussing the mode of action of air therapy and the physiological deductions, we shall summarize briefly the chief known facts about air therapy:

1: Antispasmodic action: We were led to this mode of action by the use of air in a young girl with salpingitis who had a terminal pulmonary lesion. Her attacks were featured by fever, vomiting and severe pain necessitating large doses of morphine. Pneumoperitoneum gave complete relief of pain during the injection, but the vomiting and fever continued an independent course of 7 days' duration. In a subsequent attack hysterosalpingography was done with brominol immediately before, during and after inflation, and it showed thin spastic tubes which widened to about 3 diameters at the same time as the pain was relieved and 12 cc. more of the dye was easily injected.

The same mode of procedure was used to study the barium filled gut before and after air inflation. Many cases showed complete total relief of spasm; some showed partial relief; and a few showed relief of symptoms with no relief of spasm. We will show in another paper the detailed X-ray changes and their relationship to relief of symptoms, but we may now say that about 80 per cent of all benefited cases showed partial or complete relief of spasm and about 20 per cent showed no relief of spasm. Relief of spasm was coincident with a decrease of pyrosis, nausea, distress or pain. X-ray series approximated a normal appearance from within a few minutes to a few days such as seen after months or years with light or vitamines. (See figures 2, 3, 4, 5.)

2: Although atropin and belladonna are in themselves ineffective in controlling symptoms of spasm, yet small doses have great effects when used with pneumoperitoneum. Adrenalin and ephedrin have no results on a tuberculous bowel. In tuberculous peritonitis with gastrointestinal symptoms, air alone gives variable results, but with belladonna the results are often marked in the pain and constipation.

PLATE 2

Fig. 2A. Case presenting anorexia, distention, nausea, severe cramps and constipation. X-ray shows a caecal deformity with gastric retention. (6-hour plate.)

Fig. 2B. Same case 6 weeks after first inflation. X-ray shows a normal caecum, terminal ileum, appendix and no gastric retention. Autopsy showed ulceration of caecum and valve area.

Fig. 2A

Fig. 2B

PLATE 2

PLATE 3

Fig. 3A. Case presenting symptoms of crampy pain and diarrhoea. X-ray shows a deformed caecum at all hours. (6- and 9-hour plates.)

Fig. 3B. Same case 6 weeks after first inflation. Disappearance of symptoms. X-ray shows a normal caecum at all hours. (6-hour plate.)

Fig. 3A

Fig. 3B

PLATE 3

PLATE 4

Fig. 4A. Case showing deformed caecum with enema examination immediately prior to air inflation.

Fig. 4B. Same case showing a normal caecum immediately after air inflation of 1000 cc.

Fig. 5A. Case presenting crampy pain and diarrhoea. Enema examination shows a defect involving caecum, ascending and proximal transverse colon.

Fig. 5B. Same case 9 days after air inflation of 800 cc. Disappearance of symptoms. Enema examination shows a normal colon.

Fig. 4A

Fig. 4B

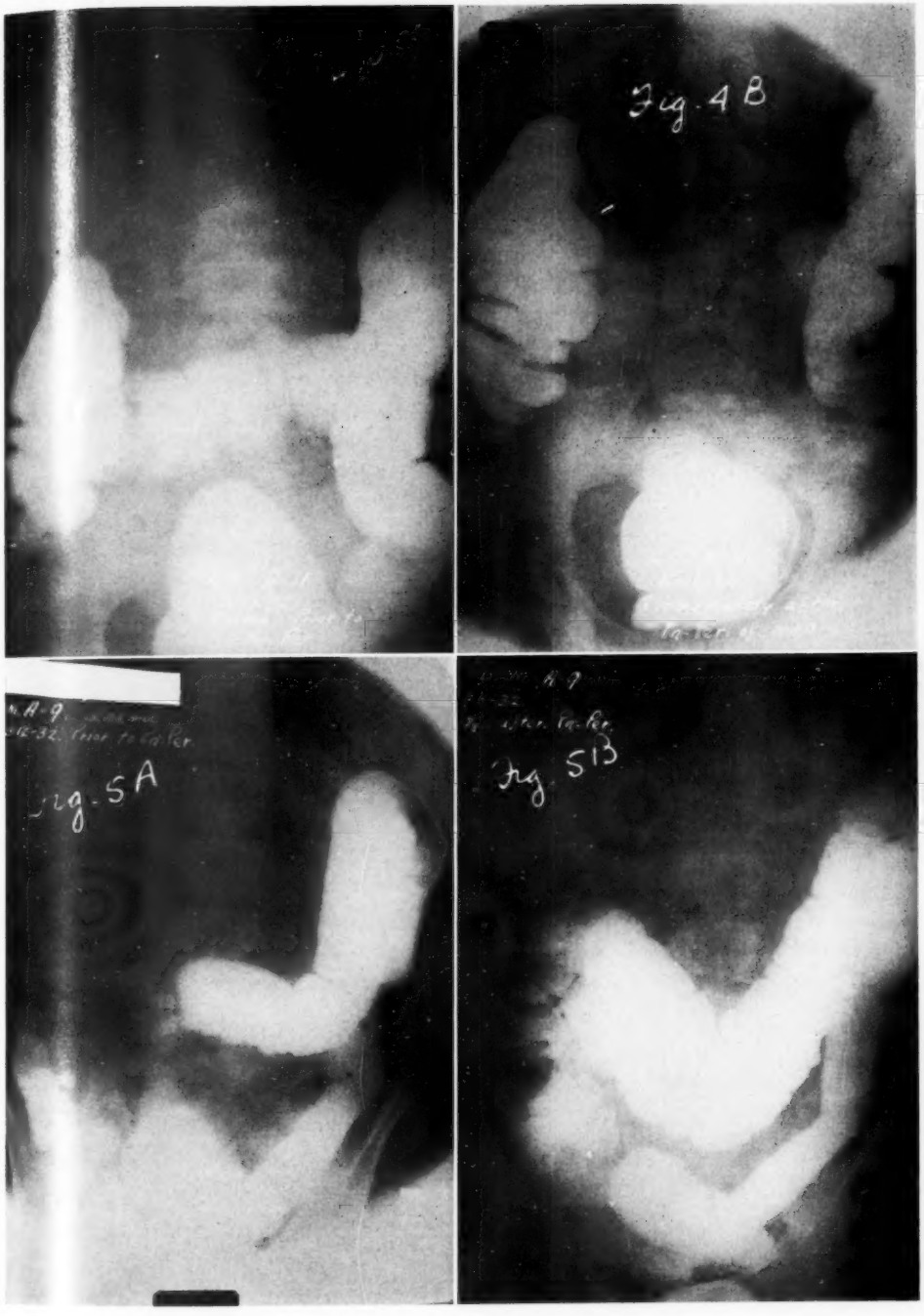

Fig. 5A

Fig. 5B

PLATE 4

3: Air therapy may give three types of relief. Immediate relief is always present and lasts as long as there is air in the abdomen. Most cases show a prolonged effect after one or two refills with good results from months to years. The symptoms may again recur after a long period and show a similar response to air therapy.

4: Postmortem observations on successfully treated cases show no greater serosal reaction, vascularity or signs of healing other than found in cases that had no treatment or only medication. The same fact is true of cases which received vitamines or light.

5: Cati (20) has shown that after successful air therapy the low content of hydrochloric acid in the stomach is increased and the gastric acidity curves assume a normal aspect. Sisti (21) has demonstrated similar changes in coprological studies with a tendency to assume normal characteristics.

PROPOSED MODE OF ACTION OF AIR THERAPY

Since most cases show rapid relief of symptoms, and X-ray changes occur from within a few minutes to a few days, the action must be neuromuscular. Rapid changes of tonus and spasm, such as seen in these cases, can occur only through nerve action. Since any gas may be used effectively and in most cases the symptoms bear a definite relationship to the absorption of the gas, the action must be mechanical. Since the gas is located intraperitoneally, it must act upon the extrinsic nerves. Since 20 per cent of the cases relieved show no signs of antispasm, then there must be an extrinsic and intrinsic nerve factor, the former being acted upon by the gas.

The mode by which the air acts upon the extrinsic nerves is problematical, and we can offer only suggestions. The intraäbdominal pressure is a negligible factor as shown in a previous paper; oxidation plays no rôle as nitrogen can be used with equal results; autopsies on successful cases show no changes in the vascularity about the ulcers, signs of healing or greater serosal reaction. It is possible that the processes of accommodation occurring in the abdomen to maintain the normal pressure may interfere with reflex processes; or that the foreign body may cause a disruption of the excessive stimuli produced; or that pressure upon the abdominal plexuses may cause an interference with reflex processes; or that the air causes rapid changes in the blood- and lymph-flow of the nerves, with a resulting detrimental effect upon them. The air acts by dampening the stimuli, and thus may be likened to a buffer.

The disruption of the reflex occurs possibly more on the afferent fibres, for atropin alone affects the extrinsic reflex slightly, but this is entirely conjectural. We wish to summarize by saying that air therapy acts by removing the extrinsic nerve reflex by a mechanical buffer action. The fact that ascites often causes an asymptomatic state in an ulcerated gut shows further the mechanical action of a fluid body in the peritoneal cavity.

APPLICATION OF THE THEORY TO KNOWN PHENOMENA

The division of the symptomatology of intestinal tuberculosis into an aetiological intrinsic and extrinsic nerve factor helps to explain many of the phenomena occurring in this disease.

If the intrinsic nerves alone are involved one may get such signs as diarrhoea and constipation, but no symptoms such as crampy pain. Nausea and vomiting might occur depending upon the degree of disturbance of the motor gradient. Pain could not occur because the pain stimuli do not reach the cord and brain. The difference between constipation and diarrhoea would then depend upon the degree of ulceration; the greater the ulceration the greater the areas of spasm and hypermotility.

The addition of the extrinsic nerve factor would add the symptoms of pain. In such a case, a diarrhoea could occur with a small lesion for the afferent-parasympathetic efferent reflex may be so intensely stimulated that the parasympathetic reaction involves adjacent segments of the gut resulting in generalized hypermotility.

In actual experience, most cases lie between these two extremes. Whether the extrinsic nerve factor is involved depends upon the local chemistry in the ulcer-bearing area and its effect upon the afferent-nerve endings and the synapses between the intrinsic and extrinsic motor nerves.

Immediate benefit with air therapy is due to a direct nervous mechanical action removing the extrinsic reflex. The good results in many of the cases after the air has been absorbed are probably due to changes in the local bowel chemistry induced by a relaxation of spasm making the neuromuscular endings less sensitive. The increased sensitivity may recur after months or years, depending upon the degree of healing the ulcers have undergone in the interim and upon the state of the focus of infection (lungs). Thus, healing takes place independently, but is assisted by a removal of spasm and relief of symptoms.

The degree of symptomatology in any one case depends upon the state of irritability of the local neuromuscular structure, the irritability of the afferent endings and the synapses between intrinsic and extrinsic motor nerves and intensity of stimulation. The degree of symptomatic severity is, then, the sum total of reflex stimulation dependent upon the intensity of the stimulation and the number of stimuli; these, in turn, depend upon the neuromuscular plus extrinsic nerve irritability and the degree of ulceration respectively. This view justifies further the classifications previously presented; for the degree of symptomatic severity is an index to the sum-total reflex stimulation; and the extent of ulceration (or, better still, the extent of physiological disturbance) is an index to the number of stimuli produced.

Thus, one may have any number of combinations between number and intensity of stimuli; and so there may be no symptoms with extensive ulceration or many symptoms with a small ulcer. The action of belladonna and atropin in cases of air therapy acts by depressing further the parasympathetic endings and the extrinsic reflex. Since adrenalin and ephedrin do not affect the tuberculous segment with or without air therapy the sympathetics apparently play no active rôle.

ACTION OF ULTRAVIOLET LIGHT AND VITAMINE THERAPY

It is known that the lamp is associated with vascular changes and that vitamines effect a generalized metabolic change, and these procedures probably act thus in relieving symptoms of intestinal tuberculosis. Whether the calcium metabolism is involved we do not know, but calcium therapy certainly produces little result. We have seen several successful cases of the lamp and vitamines, treated for a year or more with a normal-appearing bowel by X-ray, suddenly revert to their original symptoms and again show the original X-ray picture. Certainly, there was no great healing of ulcers in such cases despite symptomatic relief.

The intimate connection between ultraviolet light and vitamine metabolism leads one to suspect that they have a similar mode of action in intestinal tuberculosis. The very fact that the results of both procedures bear no relationship to the extent of the lesion but have a definite bearing upon the symptomatic severity (or sum-total reflex stimulation) points to a systemic mode of action. If they act at all, they do so regardless of the extent of the lesion. It seems possible that both act upon the inflammatory site producing a change in the threshold of sensitivity of the

peripheral neuromuscular structure. Vitamines may do so chemically through the blood-stream and light, either by changing vitamine metabolism or by changing the vascularity of the affected portion of the bowel.

PROPHYLAXIS OF INTESTINAL TUBERCULOSIS

Kantor (22) enunciates three principles in the prophylaxis of intestinal tuberculosis, namely: (1) avoid swallowing sputum; (2) give hydrochloric acid to hypoacidity cases; and (3) prevent constipation. We have not observed any great benefit from the routine administration of hydrochloric acid. The prevention of constipation and avoidance of swallowing sputum is of great importance, as it prevents the organisms from getting into the bowel and having prolonged contact with the digestive mucous membrane. It is a well-known fact that many organisms do enter the alimentary canal, especially during sleep, and the prime importance should be to prevent any organisms escaping from the lung. We have not observed any benefit from a routine administration of vitamines in the prophylaxis of intestinal tuberculosis in patients on a general diet. The prophylactic principles we have followed include:

1: A gastrointestinal X-ray series on every admission and certainly on any case of dyspepsia so that suspicious cases may be watched.
2: Treat the pulmonary lesion and make the sputum negative as quickly as possible.

TREATMENT OF INTESTINAL TUBERCULOSIS

If the case shows mild or moderate symptoms, give vitamines or ultraviolet light, together with medication, and, if the case is poorly relieved, add air therapy after three months. If the symptoms are severe, give air immediately. Every case of intestinal tuberculosis should be considered an indication for collapse therapy unless contraindicated by too extensive pulmonary disease. This applies to pneumothorax and phrenicotomy; but, prior to performing a thoracoplasty, it is well to have an asymptomatic intestinal lesion or to have the lesion well controlled with air therapy.

SUMMARY

The treatment of secondary ulcerative intestinal tuberculosis is at the present time in the same stage as pulmonary tuberculosis 10 years ago. A hopeful view may now be taken despite the absence of a specific cure,

for the various modes of therapy outlined may remove 80 per cent of the severe cases as complicating factors. Drugs and calcium are of secondary importance in themselves but highly useful with other agents; surgery has a very limited place; the diet may be individually selected. Ultraviolet light benefits 40 per cent of moderate and 15 per cent of severe cases; vitamines benefit 67 per cent of moderate and 27 per cent of severe cases; pneumoperitoneum benefits all moderate cases and 80 per cent of severe ones. A new classification is offered, based upon the roentgenological extent of the lesion and the degree of symptomatic severity. The ulcerated bowel is influenced by two factors in its production of a state of spasm or irritability, namely, a local neuromuscular reaction and an extrinsic afferent-parasympathetic efferent reflex. It is possible that light and vitamines affect the threshold of irritability of the local factor, whereas pneumoperitoneum disturbs the proper function of the extrinsic reflex. Healing of ulcers takes place by a local and general increase of immunity further benefited by release of spasm and removal of distressing symptomatology. The existing views on prophylaxis and therapy must be modified and the presence of intestinal disease be regarded as an indication for active pulmonary collapse.

CONCLUSIONS

1. The diagnosis of intestinal tuberculosis is reviewed and pneumoperitoneum is offered as a diagnostic procedure.
2. A new classifiation of intestinal tuberculosis is presented.
3. The following modes of therapy are evaluated: drugs, calcium, diet, surgery, heliotherapy, vitamines, pneumoperitoneum.
4. The pneumoperitoneum series is emphasized.
5. The influence of pulmonary collapse is stressed as a beneficial factor.
6. Additional views are offered on the pathological physiology of the ulcerated bowel in intestinal tuberculosis.
7. The mechanical buffer theory of action of pneumoperitoneum is advanced.

ADDENDUM

The detailed radiographic changes, occurring in the tuberculous bowel under pneumoperitoneum, were studied in 50 cases which had repeated X-ray examinations. In this series, good symptomatic relief resulting from air therapy occurred in 41 cases, which showed the following radiographic changes: rapid relaxation, or antispasm, of the ulcerated segment, starting from within a few minutes to a few weeks after beginning air therapy, occurred in 23 cases (55

per cent); delayed but complete relaxation occurred in 2 cases (5 per cent) within 4 and 9 months respectively; partial relaxation was present in 8 cases (20 per cent); and no change in 8 cases (20 per cent). Six cases, which showed a partial symptomatic result, presented also a partial relaxation of the involved area; and 3 failures of pneumoperitoneum therapy showed no X-ray change. Thus, in cases showing good symptomatic results, some degree of antispasm was present in 80 per cent, and none in 20 per cent. These figures present an index of the respective rôles played by the extrinsic and intrinsic nerve factors in the production of the symptoms occurring in intestinal tuberculosis.

When the cases were viewed from the radiological standpoint, the following results were obtained: good antispasm was present in 25 cases, all of which showed good symptomatic relief; partial antispasm was present in 14 cases, of which 8 (55 per cent) were associated with good relief of symptoms and 6 (45 per cent) with partial relief; no X-ray changes occurred in 11 cases, of which 8 showed good relief of symptoms and 3 were failures of air therapy. Secondary changes, such as gastric retention or generalized hypermotility, depended entirely upon the degree of relaxation of the ulcer-bearing area.

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LEUCOCYTIC COUNTS DURING BED-REST COMPARED WITH THOSE DURING EXERCISE IN TUBERCULOUS AND NONTUBERCULOUS CASES[1]

E. M. MEDLAR

One of the most important clinical tests used to determine the status of pulmonary tuberculosis is graduated exercise. It has become, through the accumulation of clinical data, an established fact that in pulmonary tuberculosis great care must be taken in the prescribing of exercise as a therapeutic measure. Through the use of carefully controlled regimens it is generally believed that the tolerance of the patient toward his disease is enhanced. The beneficial or detrimental effect of the exercise prescribed is determined by the pulse-rate, temperature, weight, physical findings, and serial roentgenograms of the chest.

In leucocytic studies on tuberculosis patients which have been in progress since 1927 in this institution, it has been noted that a number of cases have shown a change in the leucocytic picture when exercise was given. In some of the cases this change has been interpreted by the author as indicative of an unfavorable turn in the pathological process. The clinical appraisal in several instances was at variance with the pathological interpretation, since there did not occur a change in the clinical data which would suggest that the pathological process was becoming more unfavorable. Rather, these cases appeared to be definitely benefited by the graduated exercise regimen. This difference between the clinical and pathological interpretations led to a careful study of the effect of accepted sanatorium regimen on the leucocytic reaction. Moderately and far-advanced tuberculosis cases in which the sanatorium management was limited to the usual bed-rest and exercise therapy were selected for investigation. The results of the study are given below.

In previous communications (1) (2) (3) the author has submitted data which suggest that the leucocytic picture, as found in proved cases of tuberculosis, indicates the trend of the underlying pathological condition. It would seem desirable therefore to determine to what extent

[1] From the Hegeman Research Laboratory of the Metropolitan Life Insurance Company Sanatorium, Mt. McGregor, New York.

the usual sanatorium bed-rest and exercise therapy affect the leucocytic picture. In other words, how beneficial, from a purely pathological point of view, are bed-rest and graduated exercise.

To determine the influence of bed-rest upon the leucocytic picture, 50 cases of clinically active moderately and far-advanced pulmonary tuberculosis were investigated. The length of bed-rest varied from 4 months to over 2 years. A comparison was made of the leucocytic counts made during the first month of residence with an equal number of counts taken at the end of the bed-rest period, that is, 4 consecutive leucocytic counts at each period were taken from each case. These data

TABLE 1

A comparison of 4 consecutive leucocytic counts on admission with the same number at the end of of bed-rest therapy in 50 unselected cases of moderately advanced and far-advanced tuberculosis

INDEX NUMBERS	ADMISSION	END OF BED-REST THERAPY
Total Counts.....	200	200
0–10	6 (3%)	33 (16%)
11–20	22 (11%)	62 (31%)
21–30	52 (26%)	78 (39%)
31–40	51 (25%)	16 (8%)
41–50	28 (14%)	10 (5%)
51–60	15 (8%)	1 (1%)
61–70	11 (6%)	0
71–80	9 (5%)	0
81–90	3 (1%)	0
91–100	3 (1%)	0
Average index.....	36.78	20.96

are presented in table 1. In this table is compiled the distribution of the leucocytic indices. These indices, as in all subsequent tables in this article, were obtained by the use of a calculator devised by Crawford (4).

In a previous communication (3) I have shown that leucocytic indices above 30 are distinctly unfavorable in the tuberculous. An inspection of table 1 shows that during the first month of bed-rest 60 per cent of the leucocytic counts had an index above 30, whereas at the end of the period, before the patients were allowed to go out to meals, but 14 per cent of the counts were in the same range. That is, leucocytic indices above 30 were over four times more frequent at the beginning of bed-rest than at the end. This change in the leucocytic picture is also reflected

TABLE 2

A

A comparison of leucocytic counts on a group of 73 cases of clinically active moderately and far-advanced pulmonary tuberculosis at different periods during sanatorium residence

INDEX NUMBERS	PERIOD OF BED-REST		PERIOD OF EXERCISE THERAPY		
	First month	Remainder of bed-rest	Meals to 30 minutes inclusive	35 minutes to 1 hour inclusive	Over 1 hour
Total Counts.....	218	406	1,038	969	194
0–10	7 (3%)	55 (13%)	192 (19%)	186 (19%)	35 (18%)
11–20	25 (11%)	89 (22%)	168 (16%)	216 (22%)	37 (19%)
21–30	67 (31%)	125 (31%)	377 (36%)	313 (32%)	69 (35%)
31–40	52 (24%)	76 (19%)	183 (18%)	166 (17%)	39 (20%)
41–50	28 (13%)	34 (8%)	77 (7%)	51 (5%)	10 (5%)
51–60	17	17	24	25	2
61–70	9	3	8	9	2
71–80	9 } 18%	4 } 7%	5 } 4%	0 } 5%	— } 3%
81–90	3	1	2	2	—
91–100	1	2	2	1	—
Average index....	36.76	26.61	24.70	23.43	24.17
	B *Pathologically favorable on discharge (23 cases)*				
Total Counts.....	83	230	388	374	78
0–10	7 (8%)	50 (22%)	183 (47%)	174 (46%)	34 (44%)
11–20	20 (24%)	72 (31%)	102 (26%)	129 (35%)	28 (36%)
21–30	26 (31%)	63 (27%)	82 (21%)	64 (17%)	16 (20%)
31–40	18 (22%)	25 (11%)	18 (5%)	6	0
41–50	6	12	1	1	0
51–60	2	6	2	0	0
61–70	2 } 15%	0 } 9%	0 } 1%	0 } 2%	0 } 0%
71–80	1	1	0	0	0
81–90	1	0	0	0	0
91–100	0	1	0	0	0
Average index....	28.00	21.34	13.86	12.72	12.14
	C *Pathologically unfavorable on discharge (42 cases)*				
Total Counts.....	114	157	489	488	106
0–10	—	4 (3%)	9 (2%)	12 (3%)	1 (1%)
11–20	5 (4%)	17 (11%)	64 (13%)	85 (17%)	9 (9%)
21–30	39 (34%)	55 (35%)	233 (47%)	237 (48%)	53 (50%)
31–40	30 (26%)	49 (31%)	126 (26%)	126 (25%)	33 (31%)
41–50	20 (18%)	19 (12%)	45 (9%)	25 (5%)	8 (7%)
51–60	10	8	6	2	2
61–70	4	3	3	1	0
71–80	3 } 18%	1 } 8%	0 } 3%	0 } 2%	0 } 2%
81–90	2	0	1	0	0
91–100	1	1	2	0	0
Average index....	38.48	32.42	29.29	27.06	29.36

TABLE 2—*Concluded*

D

Pathologically very unfavorable on discharge (8 cases)

INDEX NUMBERS	PERIOD OF BED-REST		PERIOD OF EXERCISE THERAPY		
	First month	Remainder of bed-rest	Meals to 30 minutes inclusive	35 minutes to 1 hour inclusive	Over 1 hour
Total Counts.....	21	19	111	127	10
0–10	—	1 (5%)	—	—	—
11–20	—	—	2 (2%)	2 (2%)	—
21–30	2 (9%)	7 (37%)	12 (11%)	12 (9%)	—
31–40	4 (19%)	2 (10%)	39 (35%)	54 (42%)	6 (60%)
41–50	2 (9%)	3 (15%)	31 (28%)	25 (19%)	2 (20%)
51–60	5	3	16	23	—
61–70	3	—	5	8	1
71–80	5 } 63%	2 } 33%	5 } 24%	— } 28%	— } 20%
81–90	—	1	1	2	1
91–100	—	—	—	1	—
Average index....	53.12	42.31	43.32	43.57	43.56

in the average leucocytic index, which shows a drop of over 16 units (43 per cent) as a result of this means of therapy. This shows that from the angle of the pathological activity the group as a whole has been greatly benefited.

It is of equal importance to determine whether graduated exercise brings about a further change in the leucocytic picture. For this purpose 73 clinically active cases of moderately and far-advanced tuberculosis were investigated. These cases had all been discharged from the sanatorium. To compare different stages in the sanatorium residence, the bed-rest period was divided into two periods: the first month and the remainder of the period; while the period of graduated exercise was divided into three groups: meals to 30 minutes inclusive, 35 minutes to one hour inclusive, and over one hour of exercise. In table 2A the distribution of the leucocytic indices and the average leucocytic index for the group as a whole for each of the above periods is given. In all, there were 2,825 counts on the 73 cases.

The percentages of leucocytic indices above 30 for the five columns in table 2A are 55 per cent, 34 per cent, 29 per cent, 27 per cent and 28 per cent, respectively. One gains the impression that there has occurred in the group as a whole a very significant improvement in the pathological

process during the period of bed-rest therapy, while during the period of graduated exercise a further significant improvement has not occurred. The same phenomenon is reflected in the average leucocytic index for each column. The average leucocytic index of 24 on discharge is significantly higher than the average of 15 (3) for normal persons. From this one may judge that this difference is due to the pathological tuberculous process, and, because the index is higher in the tuberculous group, the pathological changes of the group as a whole are unfavorable at the time of discharge, though they are much more favorable than on admission. In other words, the pathological process has been but partially controlled as the result of the sanatorium treatment.

From both the clinical and the pathological viewpoints it is generally recognized that the severity of the *disease* tuberculosis varies greatly within a group of cases having the same extent of disease. Consequently, when any group of tuberculous cases is considered as a whole it is quite possible that the status of subdivisions of the group is masked. To determine whether this were true the whole group was divided into favorable, unfavorable and very unfavorable pathological groups (3) on a basis of the last 6 consecutive counts before discharge.

On the above basis the group of 73 cases was found to be composed of 23 favorable, 42 unfavorable and 8 very unfavorable cases. In tables 2B, C and D are given the distribution of leucocytic counts and the average leucocytic indices in a manner similar to table 2A.

In the favorable groups (table 2B) it will be noted that in the 5 columns the percentage of leucocytic indices above 30 has been reduced as follows: 1, 37 per cent; 2, 20 per cent; 3, 6 per cent; 4, 2 per cent; and 5, 0 per cent. This phenomenon is also manifest in the average leucocytic index, where in column one it is 28 and in column five it is 12. From this one may judge that the pathological process has improved even when graduated exercise was instituted. It is of interest that during the first month of residence the average leucocytic index per case varied from 7 to 69. In other words, on entrance to the sanatorium the cases in this group ranged all the way from pathologically favorable to very unfavorable.

The unfavorable group (table 2C) shows that as a whole they were pathologically worse than the favorable one on admission and that they failed to show as great a pathological improvement. That a certain degree of improvement occurred is reflected in the percentage of leucocytic indices above 30 in the five columns. They are: 1, 62 per cent; 2, 51 per cent; 3, 38 per cent; 4, 32 per cent; and 5, 40 per cent. The

average leucocytic index also shows that the degree of pathological improvement in the two groups is quite different. In the favorable group there was a decrease of 57 per cent in the average leucocytic index whereas the greatest decrease in the unfavorable group was 29 per cent. In the unfavorable group the average leucocytic index per case during the first month of residence ranged from 22 to 65. There were no cases pathologically favorable on admission and no case stabilized within a favorable pathological range during sanatorium residence whether on bed-rest or exercise therapy. In this group there were cases which showed marked improvement in the leucocytic picture during bed-rest and then on exercise the leucocytic reaction reverted to that present on admission. Some of these cases showed no clinical reactivation of their disease while in the sanatorium.

In the very unfavorable group (table 2D) the pathological severity is found to be the worst and the improvement the least. What improvement did occur appears to be entirely due to bed-rest therapy. This is shown in the percentage of leucocytic indices above 30 for the 5 columns, as follows: 1, 91 per cent; 2, 58 per cent; 3, 87 per cent; 4, 86 per cent; and 5, 100 per cent. During the first month of residence the average leucocytic index per case ranged from 42 to 85. They were all very unfavorable.

From the above analysis it has been demonstrated that from a pathological point of view, the group as a whole is made up of subgroups in which there were different degrees of severity of the tuberculous involvement. When the records of individual cases were examined, it was also found that while all showed pathological improvement, especially during the period of bed-rest, the degree of improvement varied greatly even under the same regimen. Thus, with two individuals having an average leucocytic index of 65 on admission, one would have an average index of 15 and the other of 45 on discharge. It was also found that there was no uniformity of behavior in the leucocytic picture during the period of graduated exercise. In some individuals the leucocytic picture continued to improve, in others it remained practically stationary, while in some it became distinctly worse. There was not always an agreement between the trend of the leucocytic picture and the clinical appraisal of the cases.

This group is too small to warrant a careful statistical analysis of what has happened to the cases since discharge from the sanatorium. A brief comparison of the favorable and very unfavorable groups is, however,

of interest. Of the 23 cases in the favorable group there have been no deaths, one has had a clinical relapse, two are continuing the "cure," and the remaining 20 have remained at work. The records show that in the very unfavorable group (8 cases) 2 have died of tuberculosis, 4 have had clinical relapses and 2 have remained at work.

Because the pathological interpretation and the clinical appraisal of tuberculosis cases were at variance at times, it was decided to make a very detailed study of the leucocytic picture in a small group of tuberculous cases and to use as a control for this study a small group of nontuberculous patients.

The clinical staff chose for the study a group of 5 moderately advanced tuberculous cases that had recently entered the institution for treatment. As a control, 6 nontuberculous cases, that were as nearly normal as could be obtained from a group of over 100 nontuberculous patients, were selected. The principal diagnosis of the 6 nontuberculous cases was as follows: (1) fibrosis of myocardium and hypertrophied prostate; (2) chronic rhinitis and secondary anaemia; (3) chronic cervical lymphadenitis, secondary anaemia and minimal tuberculosis (of no clinical significance); (4) anxiety neurosis and migraine; (5) psycho-neurosis, hypochondriasis and visceroptosis; and (6) myxoedema and bronchial asthma. From this list of diagnoses it can be seen that these persons cannot be considered as normal healthy persons. None were, however, seriously ill clinically. The group of tuberculous cases were not seriously ill with their disease, as judged by the clinical staff. Since the groups selected for study were not seriously ill clinically may it not be assumed that, when placed under the same regimen, they should present a similar leucocytic picture, unless the pathological changes present affected the leucocytic reaction?

The general procedure for the investigation was as follows. Those of the nontuberculous group were to be strict bed cases for at least 3 weeks after the study was begun. When exercise was allowed it was to be increased as rapidly as possible, due consideration being given to the welfare of the patient in the increase of the exercise period. The tuberculous cases were all to be strict bed cases for a month. After the expiration of this period the allowance and graduation of exercise was left to the discretion of the physician responsible for the case. Careful and frequent clinical check-ups of all the cases were to be made. The laboratory was to remain ignorant of the clinical procedures during the study.

On the laboratory side, daily leucocytic counts were to be made throughout the study. When exercise was begun, leucocytic counts were to be made within half an hour after the exercise period, as well as the usual daily count previous to exercise, as long as the director of the laboratory deemed it advisable. The obtaining of the blood samples was to be placed in the hands of one competent assistant for the entire study. This was adhered to with but few exceptions. Total and differential leucocytic counts were to be made on all blood samples. This part of the work was apportioned to three competent laboratory assistants. The clinical staff was to remain in ignorance of the results of the laboratory findings until the end of the study.

A total of 2,109 leucocytic counts was done, entailing a total of 1,722 days of observation. The shortest period of observation on a nontuberculous case was 59 days and on a tuberculous case 179 days. The longest period of observation on a nontuberculous case was 114 days and on a tuberculous case 363 days. The average length of observation on the nontuberculous cases was 85 days and on the tuberculous 242 days.

All of the nontuberculous cases were permitted an hour of walking twice daily within a month after exercise was begun. None of the tuberculous cases was allowed this amount of exercise within four months after exercise was begun.

In the nontuberculous cases a few incidents of clinical significance occurred during the study. One case had several asthmatic attacks. On one occasion food poisoning caused a mild gastrointestinal upset. An occasional mild upper respiratory infection occurred. Similar conditions existed among the tuberculous cases except that there were no asthmatics among them. One tuberculous case showed an increase by roentgenogram during the study. Otherwise, from the clinical viewpoint, all of the patients progressed satisfactorily.

An analysis of the data on the leucocytic counts was made relative to the effect of exercise on the leucocytic reaction as compared to the leucocytic picture during the period of bed-rest. The tuberculous and nontuberculous groups were tabulated separately for comparison. Since it was the effect of exercise rather than the chance happening of "colds," etc. that was the point of prime interest in the study, a complete analysis was not attempted relative to the correlation of shifts in the leucocytic picture with the occurrence of clinical manifestations.

In table 3 is given a compilation of the range and average for the total count: the percentages of neutrophiles, lymphocytes and monocytes;

TABLE 3

A comparison of range and average of daily leucocytic counts in 5 tuberculous and 6 nontuberculous cases

NUMBER OF COUNTS	PERIOD OF BED-REST AND OF EXERCISE								BEFORE AND AFTER EXERCISE							
	Nontuberculous (6 cases)				Tuberculous (5 cases)				Nontuberculous (6 cases)				Tuberculous (5 cases)			
	Bed		Exercise		Bed		Exercise		Before		After		Before		After	
	204		306		409		803		289		289		98		98	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Total counts in thousands	5–12	8,138	4–16	7,983	4–15	8,251	4–17	7,906	4–16	7,988	5–16	8,531	5–17	8,898	5–18	9,180
Polymorphonuclears—per cent	46–90	59.9	33–73	55.3	45–90	58.1	41–83	57.4	33–73	54.8	31–77	54.4	47–68	55.1	45–70	56.1
Lymphocytes—per cent	16–43	27.7	17–58	30.7	6–47	31.0	12–47	29.7	12–59	30.9	13–57	31.6	17–38	30.0	16–36	28.7
Mononuclears—per cent	4–14	8.5	4–14	9.0	4–27	9.8	4–26	8.8	4–13	9.0	4–12	9.4	4–12	7.8	4–14	8.8
Leucocytic index	7–48	22.1	0–56	17.9	0–67	18.8	0–74	18.0	0–52	17.5	0–64	16.9	0–30	13.8	0–30	17.1

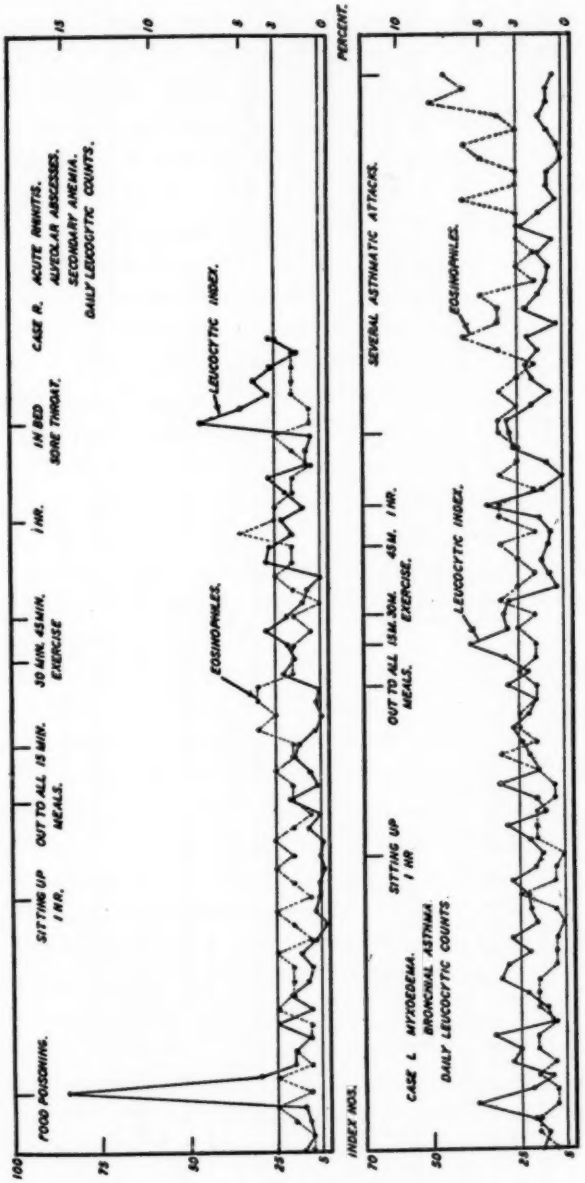

CHART 1. EFFECT OF EXERCISE—NONTUBERCULOUS CASES R. AND L.

(For description see text, p. 484.)

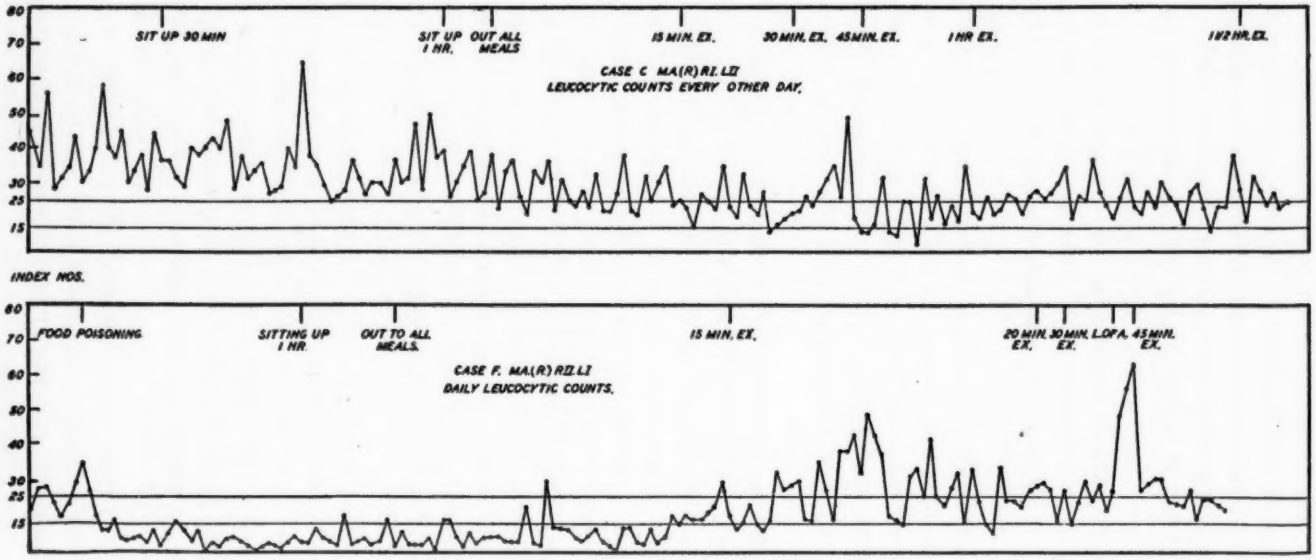

CHART 2. EFFECT OF EXERCISE—TUBERCULOUS CASES C. AND F.

(For description see text, p. 485.)

and the leucocytic index for the tuberculous and nontuberculous groups. The data representing the period of bed-rest are compared with those obtained during the period of graduated exercise. Also the data on the counts taken before and within half an hour after the morning exercise period are compared.

A study of the data in this table shows the following points of interest. In both groups the average total counts are slightly but not significantly lower during the exercise period than during that of bed-rest. The immediate effect of exercise is to cause an insignificant rise in the total counts. Gradual increase of exercise does not appear to have caused a significant change in the total leucocytic count when the groups as a whole are considered.

The greatest change in the differential counts lies in the average neutrophile and lymphocyte percentages. Here it will be noted that, in comparing the period of bed-rest with that of exercise, the nontuberculous cases show a drop in the neutrophile and a rise in lymphocyte percentage, whereas in the tuberculous group the drop in neutrophiles is considerably less and, instead of a rise, there is a decrease in lymphocytes. The data also show that the immediate effect of exercise on the average neutrophile and lymphocyte percentage in the nontuberculous cases is nil, whereas there occurs a slight rise in the neutrophile and a slight drop in the lymphocyte percentage in the tuberculous cases. The possible significance of this difference between the tuberculous and nontuberculous cases will be discussed later.

The nontuberculous group shows a decrease in the mean leucocytic index during the period of exercise and no change relative to the immediate effect of exercise. The tuberculous cases reveal no significant change in the average leucocytic index between the period of bed-rest and of exercise, whereas the immediate effect of exercise is to bring about a slight rise.

A study of the individual cases showed that all did not react in the same way, and that the groups, when taken as a whole, tended to nullify the changes in individuals. To illustrate the difference in individuals the leucocytic index graphs for two nontuberculous cases, L. and R., and two tuberculous cases, C. and F., are given in the charts. The main points of interest are indicated in each chart. In the nontuberculous charts the index numbers are given on the left and the eosinophile percentage on the right. Two horizontal lines are shown. Between these two lines is the normal variation of the leucocytic index for normal

healthy persons. The upper line also indicates the upper normal limit for eosinophile percentage. The percentage of eosinophiles exceeded the normal limit fairly consistently over the period during which asthmatic attacks occurred. It will be noted that in case R. the index increased and in case L., in spite of the asthmatic attacks, the index decreased during the period of exercise. Case R. was the only nontuberculous case which showed a rise of the leucocytic index when on exercise.

In the charts of the tuberculous cases C. and F., two horizontal lines are shown. Below the line at 15 the pathological interpretation is favorable. All index numbers above the line at 25 are interpreted as pathologically unfavorable, the larger the more unfavorable. The indices between 15 and 25 are interpreted as slightly favorable or slightly unfavorable, depending upon which line they approach. The index numbers are indicated at the left of the charts. The graph of case F. indicated a distinctly unfavorable pathological trend during the period of exercise as compared with a distinctly favorable picture during bed-rest. This case gave no clinical evidence that such a change was occurring in the leucocytic picture. In the tuberculous group, 2 showed a drop and 3 a rise of the leucocytic index during the period of graduated-exercise therapy.

In all the graphs the dots represent the periods of leucocytic counts. To reproduce the whole period of observation on case C., counts for every other day are shown. In all of the other cases daily counts are shown.

DISCUSSION

Tuberculosis is a disease which is generally recognized as being so insidious in its progress that a serious pathological process may become definitely established before the disease becomes clinically manifest. In other words the subclinical stages are as important, if not more so, than the periods when clinical symptomatology becomes evident. This is so generally recognized that at the present time the X-ray, which is in reality an impersonal scientific test in which shadows are determined, is regarded as the most important single examination method available to the clinician (5).

As a matter of fact any test which will lead to a more accurate understanding of the type of pathological activity present in a tuberculous individual who is in a subclinical stage of the disease should prove of distinct value. The author in previous communications has presented arguments relative to the use of leucocytic counts, interpreted upon a

pathological basis, as an additional aid to the clinician in the evaluation of tuberculosis. If the arguments set forth in those articles prove to be logical and valid then that test should be of importance when subclinical tuberculosis is present.

The latter part of the data presented above represents an intensive study in a small group of tuberculous and nontuberculous individuals of the leucocytic picture, day by day, as the clinical regimen was gradually changed from complete bed-rest to a considerable amount of exercise in the form of walking. Such a study is of importance, since one of the most serious problems in a rehabilitation program for the tuberculous is the establishment of the amount of work which such persons can undertake without too serious an effect upon their disease.

One of the most important facts uncovered in the study was that all individuals do not show the same type of change in the leucocytic picture when the status is changed from rest to exercise. Also that even if the trend of the reaction is the same it may differ in the degree of change. This lack of uniformity in the shift of the leucocytic picture naturally leads to the conjecture that in some patients exercise may have a beneficial influence while in others the reverse may be true. If this phenomenon should also be found to exist in a larger series of cases, as is shown in tables 1 and 2, then may it not be possible to individualize the cases and determine the fitness of a patient for exercise? If this should be found to be possible, then an additional and valuable test would be available to aid in the establishment and management of the therapeutic exercise periods for tuberculous cases.

The one factor of greatest significance in the leucocytic picture of tuberculous cases is the ratio between the neutrophiles and the lymphocytes. The reason for this ratio being of such great importance is that in the tuberculous lesions the neutrophiles represent one type (unfavorable) of pathological activity and the lymphocytes another (favorable). The factors of the total count and of the monocyte percentage are also important, as they complete the leucocytic picture, but when taken by themselves they are not as important as the neutrophile-lymphocyte ratio. It is interesting to note the difference between the neutrophile-lymphocyte ratio in the nontuberculous and tuberculous groups as shown in table 3 when the rest and exercise periods are compared. In the nontuberculous group this ratio is *reduced* from 2.16 at rest to 1.79 at exercise, whereas in the tuberculous group it is *increased* from 1.80 at rest to 1.93 at exercise. Considering the fact that the amount of exercise was increased much more rapidly in the nontuber-

culous than in the tuberculous cases, the opposite trend has an increasing significance. If it had been possible to have instituted a much more rapid increase of exercise in the tuberculous cases it seems plausible that this divergence of the neutrophile-lymphocyte ratio would have been more striking. In any event the data herein given show that with exercise the nontuberculous group had a rise of lymphocytes at the expense of neutrophiles whereas the reverse was true in the tuberculous group.

A summation of the entire leucocytic picture brought out another interesting fact, namely, that the immediate effect of exercise was to increase the leucocytic index in tuberculous cases whereas in the nontuberculous cases a decrease was found. This change can hardly be of real significance since it occurs in such a short time. It probably indicates that a somewhat more accurate picture of the actual leucocytic content of the blood is obtained as a result of a more rapid circulation and a better mixture of the cellular content of the blood.

A fact of considerable importance was that two out of the five tuberculous cases showed a much greater rise of the leucocytic index during the exercise period than did any of the other cases studied. This is easily seen when the graph of case R., the nontuberculous individual who showed an increase of the index, is compared with case F., one of the tuberculous cases that showed a considerable increase of the index during the exercise period. In case R., 10 out of the 31 counts taken during the exercise period exceeded the limit for normal persons and, of these, 5, or one-half, could be accounted for by the fact that an acute upper respiratory infection developed. In contrast to case R., case F. shows that 38 out of the 72 counts taken during the exercise period exceeded the limit for normal persons. There was nothing of a clinical nature that could explain this change.

A comparison of the leucocytic graphs of cases R. and F. during the period of bed-rest is of interest. In case R., 3 out of 28 counts passed above the normal limit. These were all explained by the fact that a gastrointestinal upset occurred as a result of food poisoning. In case F., 7 out of 100 counts passed above normal limits. Of these, three can be explained on the basis of food poisoning. There is a striking contrast between the counts at bed-rest and those during the exercise period in this latter case. What has brought about this change? Is it caused entirely by the change in regimen from rest to exercise? If so, is it not logical to expect that both cases should have shown an equally great shift in the leucocytic picture?

In complex biological phenomena it is often difficult to prove that the

effect observed is due to a specific cause. If a person is in an accident and a bone is found broken, it is sound logic to reason from cause to effect and say that the accident was responsible for the broken bone. If an individual ingests a large amount of sugar and a definite increase in blood-sugar can be demonstrated afterward, then it is reasonable to conclude that the rise in blood-sugar is a result of the absorption of the large sugar intake. If a person is clinically ill and lobar pneumonia can be detected, then it is sound logic to reason that the high total and high neutrophile percentage found in the leucocytic picture are caused by the pneumonic process. If, however, an abnormal leucocytic picture is found in a person in whom no definite clinical manifestations of disease are present then the interpretation becomes difficult to prove even if it is correct. Here the cause may be so obscure, hence subclinical, that the effect observed is difficult to connect directly with the cause. This is the problem presented in the graphs on case R. and F. Since the status of these persons was changed from bed-rest to exercise and the change in the leucocytic picture came with the advent of exercise, then one might argue that exercise is the cause and the leucocytic change the effect. However, other individuals on the same regimen showed the opposite effect. To be logical one must then conclude that exercise can cause opposite effects in different individuals. Can the same cause produce opposite effects in the leucocytic picture? If so, then is it not important to know what effect is being produced? This is well shown in the analysis made in table 2 of 73 cases of moderately and far-advanced tuberculosis. It seems plausible that exercise might affect the pathological process in tuberculous cases and that whatever effect is produced might be reflected in the leucocytic picture.

It is the opinion of the author that the main function of leucocytes is to dispose of products harmful to the normal functioning of living tissue. Such products are probably being produced during the normal processes of life in the state of health. They may be increased by the advent of many infectious or noninfectious conditions. Such processes may not be of a magnitude sufficient to produce clinical illness, but the absence of clinical illness and its resultant incapacitation does not argue against the presence of an abnormal state being present within the body. Hence the finding of abnormal leucocytic reactions without accompanying clinical manifestations should not relegate such findings to the realm of insignificance. The further medical knowledge can penetrate into the fog of subclinical disease the greater should be the opportunity to avert impending catastrophies.

The X-ray, which is now accepted as a most useful tool by clinicians, is an excellent example of an entrance into the field of subclinical disease. In the beginning, scepticism was rife relative to the occurrence and significance of the shadows revealed by the X-ray film. This was so because the findings were so often at variance with other clinical manifestations. To-day no one questions an increase of pulmonary tuberculosis when shown by X-ray films even though there may be no other clinical symptom to substantiate it. May it not be possible that leucocytic reactions offer an added means of estimating the subclinical phases of this disease? May it not be found to signify as much within its own field as does the X-ray now? If so, it should stand upon its own merits, as does the X-ray, without having to agree with the usual clinical manifestations of disease.

To the author the change or lack of change in the leucocytic picture occurring during the period of exercise regimen in tuberculous cases merely reflects the effect produced upon the pathological process. In the cases studied it appears that exercise may have either a beneficial, or a detrimental effect, or it may have no effect at all. If the effect is such that a more unfavorable leucocytic reaction is produced then much greater care should be used in the prescribing of exercise than if its effect is contrariwise. If this be true, then time should prove that a more intelligent use of graduated exercise in tuberculous cases can be attained. Experience may show that, by the use of leucocytic counts, tuberculous cases may be handled more scientifically as individual problems.

With present knowledge relative to the treatment of tuberculous cases the author does not believe it to be either feasible or sensible to withhold exercise from individuals because of unfavorable tuberculous pathological changes. Were this done, many a tuberculous case would never get out of bed. In fact, too great confinement may have as detrimental an effect upon the pathological process as would too great latitude in activity. However, there are instances when individuals may be deprived of the opportunity to cure their tuberculosis unless prolonged bed-rest is prescribed. Such cases can be selected by the change brought about by bed-rest therapy and the effect subsequent exercise may produce on the leucocytic reaction. It would seem a bit unfair to an individual to change from bed-rest to exercise at a point where he was just beginning to gain the upper hand of his disease and by the change in treatment throw the delicate balance against him. It is in just such situations that the leucocytic reaction may be of tremendous import, though it may not coincide with clinical appraisals.

It is realized that the size of the groups included in this study is small.

In such a study one is confronted with a choice of a large group with infrequent observations or smaller groups with frequent observations. For this study the latter choice seemed preferable.

CONCLUSIONS

1. A comparison of 200 consecutive leucocytic counts on 50 cases of moderately and far-advanced tuberculosis at the beginning of bed-rest is made with a similar number at the end of such therapy. A marked improvement of the pathological process, as reflected in the leucocytic reaction, was found to have occurred.

2. An analysis of 2,825 leucocytic counts on 73 cases of moderately and far-advanced tuberculosis during the entire period of sanatorium residence showed the following points of interest: (a) all cases improved pathologically during bed-rest; (b) the degree of improvement varied markedly in individual cases; and (c) on graduated exercise, pathological improvement continued in some cases, while in other cases there was no evidence of further improvement and in some cases the pathological process became worse.

3. An intensive study of daily leucocytic counts on 5 tuberculous and 6 nontuberculous cases is presented. A total of 2,109 leucocytic counts and 1,722 days of observation were made. The average length of study was 85 days on the nontuberculous and 242 days on the tuberculous group.

4. All the cases did not show the same trend in the leucocytic reaction during the exercise period. The pathological significance of this lack of uniformity is discussed.

5. Arguments relative to individual management of tuberculous cases, by use of leucocytic counts, in the prescribing of graduated exercise are presented.

Special technical assistance was given the author by E. G. Mullen and E. Tibbets in the study of daily leucocytic counts in the tuberculous and nontuberculous groups. Dr. A. M. Crawford largely controlled the management of the cases in this part of the study. The author desires to express his appreciation of the interest and criticisms of the clinical staff and of the careful technical assistance rendered by the laboratory staff that made this study possible.

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BILATERAL ARTIFICIAL PNEUMOTHORAX IN THE TREATMENT OF PULMONARY TUBERCULOSIS

H. FRANK CARMAN[1]

Pneumothorax has attained an important and indispensable place in tuberculosis therapy, but it is impossible to use it without a thorough understanding of the haemodynamics and pneumodynamics of the cardiorespiratory system. In administering bilateral pneumothorax it is much more important that the operator give even more consideration to the physiology of respiration and its relation to the cardiovascular system. Bilateral collapse is more than twice as difficult to carry out as unilateral collapse.

When a lung is kept at negative pressure, say minus 2 to minus 4 mm. of water, there is little change in the cardiorespiratory system. The decrease in blood-flow through the compressed lung is compensated for by a speeding-up of the blood through the uncompressed lung tissue and through the contralateral lung. This compensatory balance is maintained even to zero pressure. As positive pressure in the pleural space is increased, in the absence of adhesions, the lesser circulation becomes more and more embarrassed and more strain is thrown on the right ventricle of the heart. It will be noted that dyspnoea increases with increased positive pressure. When a bilateral collapse is produced and a moderately high negative pressure is maintained on both sides, the physiological balance between the circulatory and respiratory systems is kept and no dyspnoea is noticed. This balance is maintained in three ways: first, by compensatory emphysema of normal lung tissue on both sides; second, by speeding up of the flow of blood through the uncompressed lung tissue; and, third, by increase in the sluice of blood through capillaries.

At a tuberculosis congress in Rome in 1912 Maurizio Ascoli made the statement that it was not necessary to carry a patient through pneumothorax with a high-pressure collapse. He no doubt had made a thorough study of the physiology of respiration and had a fair knowledge of pneu-

[1] Dallas, Texas.

modynamics and haemodynamics. It had been his observation that the majority of patients did as well or better under a low-pressure reading than under high. With this in view he went further to state that a patient could be given bilateral collapse when it became necessary to treat tuberculosis in both lungs and that the best therapeutic results were had when the lung, whether one or both, was kept at that "correct compression." This was a new idea and contrary to the teaching of Forlanini who, for the last sixteen years, had maintained that it was necessary to produce high-pressure pneumothorax to obtain results. He had taught that if the lung was not completely compressed the pathological process would be aggravated rather than arrested. With his idea in view bilateral pneumothorax would be impossible.

When a normal lung, in the absence of pleuritic adhesions, is compressed, the pneumothorax will be uniform. The presence of adhesions will, of course, many times spoil an otherwise perfect compression. It has been noted that the greatest degree of collapse is so often over the affected area, giving a selective collapse. This is produced in three ways: first, by the decreased elasticity of the infected lung tissue and retraction from scar-tissue in and around the area of involvement; second, compensatory emphysema of normal lung tissue; and, third, increased use of normal lung tissue forces the air in the pleural space over the affected area and gives the greatest degree of compression. This is a type of collapse we all like to obtain, particularly in bilateral pneumothorax.

Before bilateral pneumothorax is instituted there are several things to be considered: first, the physician should at least be thoroughly competent to carry a patient through unilateral pneumothorax. If he can not intelligently administer the treatment on one side, he certainly can not administer it on both. The difficulties ahead with bilateral collapse are sometimes vast and intricate. A great number of patients who are started on unilateral collapse should be considered candidates for a double collapse, either successive or simultaneous. Second, the operator should have a thorough understanding of the physiology of the cardiorespiratory system. Third, the patient's vital capacity must be studied. When it is below 2000 cc. a double collapse should not be instituted except in an emergency. When 500 cc. of air are placed in each pleural space it means that the patient has lost 1000 cc. of breathing capacity. The cardiorespiratory system must compensate for this loss. It is better not to refill both sides on the same day, although it can be done. In one case

which will be reported dyspnoea was experienced if 50 cc. too much air were given. An effort is made to keep always that "correct pressure." Fourth, the selection of cases is a very important consideration. It is just as important to know when not to institute collapse therapy as it is to know when to use it. A careful study should be made of the pulmonary pathology, and extrapulmonary complications should be considered if there be any.

INDICATIONS FOR BILATERAL PNEUMOTHORAX

1: Limited progressive bilateral disease without cavitation
2: Bilateral cavitation, preferably the moth-eaten type
3: Reactivation of trouble in the contralateral lung
4: Uncontrollable haemoptysis in the opposite lung
5: Occasionally pleurisy, with effusion in the opposite pleural space
6: Acute tuberculous pneumonia when the process is not too acute and extensive.

CONTRAINDICATIONS FOR BILATERAL PNEUMOTHORAX

1: Extensive bilateral lesions with or without cavitation
2: Large thickened wall cavities that resist compression
3: Extensive fibroses in both lungs
4: In successive bilateral pneumothorax when a large cavity in the initial lung resists compression
5: Extensive pleuritic adhesions. A satisfactory collapse can rarely be obtained in the presence of this difficulty
6: Low vital capacity, below 2000 cc.
7: Patients above forty or forty-five years of age. After one passes forty the cardiorespiratory system fails to compensate as well
8: Extensive extrapulmonary complications. Tuberculous laryngitis is not a contraindication. It is usually very materially helped by stopping the cough
9: Threatening cardiac decompensation
10: Extremely low vitality and marked toxaemia.

In view of the above it is apparent that bilateral pneumothorax should give the best results when instituted as early as possible. Later in the disease it is not only likely that adhesions, thickened walled cavities and fibroses may interfere with proper collapse, but it is also probable that the best results cannot be obtained without interference with pulmonary and cardiac function.

Most cases of bilateral pneumothorax are carried on successively rather than simultaneously. One lung is collapsed several weeks or months

longer than the other. When the opposite lung is finally collapsed it is carried on simultaneously. It is both surprising and gratifying how much trouble will heal in the contralateral lung when the one more involved is collapsed. This saves us many times from doing a double collapse. There are certain cases with basal lesions which will not respond to pneumothorax alone and on which a phrenicectomy should be done as a supplementary help.

Regardless of the method of treatment instituted, whether bed-rest or surgical procedure, it is imperative that the physician have absolute coöperation of both patient and family. It is not infrequent that, after

Case Reports

Case 1: Mrs. O. R. W., married, age 23, came under my observation January, 1931. The family history was negative for tuberculosis. Past history was negative save for an acute attack of appendicitis at the age of 14. She has had an appendicectomy since she came under my care. Her present disability dates back to 1930 at which time a diagnosis of tuberculosis was made, and she was sent to the State Sanatorium. After nine months of hospitalization she entered training as a nurse. The latter part of December, 1930, she had an exacerbation of her tuberculous infection. When she came under my care there was a tuberculous involvement of half of the left lung as shown by figure 1, January, 1931. The right lung was practically normal. The sputum was positive for tubercle bacilli. Pneumothorax was begun in February, 1931, on the left side, when 550 cc. of air were given at the first injection. There was no difficulty experienced in getting a compression. She was free of symptoms in eight to ten weeks. Difficulty was encountered in getting her to rest enough. After the first two months she became ambulant. In May, 1934, there was an extension of the infection into about half of the right lung. Bed-rest was again advised which caused the symptoms to abate for a while. Pneumothorax was continued on the left. It was observed in July of this same year that the right lung was becoming more involved with beginning cavitation, shown by figure 2. Pneumothorax was started on the right side immediately. Figure 3 which was made in September shows a selective collapse on both sides with a cavity partially open in the right lung. Since this film was made the cavity has entirely closed. She has married and with care is leading a normal life.

pneumothorax is instituted, whether unilateral or bilateral, and the toxic process abates, the patient becomes too ambitious. With increased activity it will be found that he is using more air from each pleural space and, if not carefully watched, one or both lungs may reëxpand, forming effusions and troublesome adhesions.

The operator should know at all times the degree of compression in both pleural spaces. He cannot possibly do this without the constant use of the fluoroscope and also the roentgen ray films when needed. It is impossible to tell the amount of compression with the stethoscope. As both lungs are compressed, the patient will be inclined to use more air

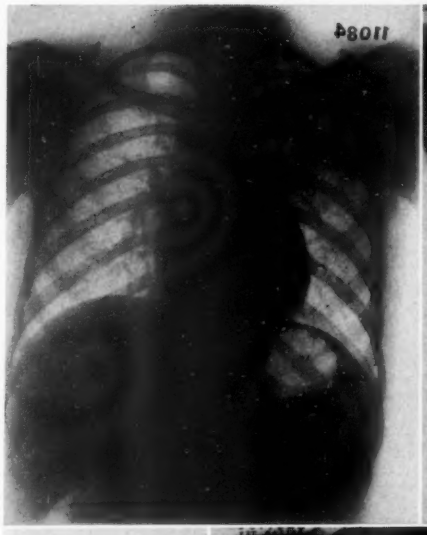

Fig. 1

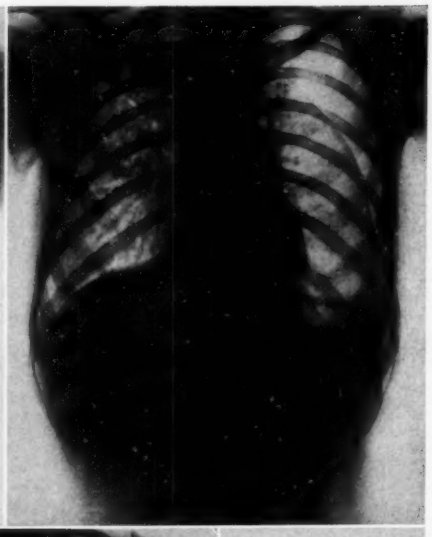

Fig. 2

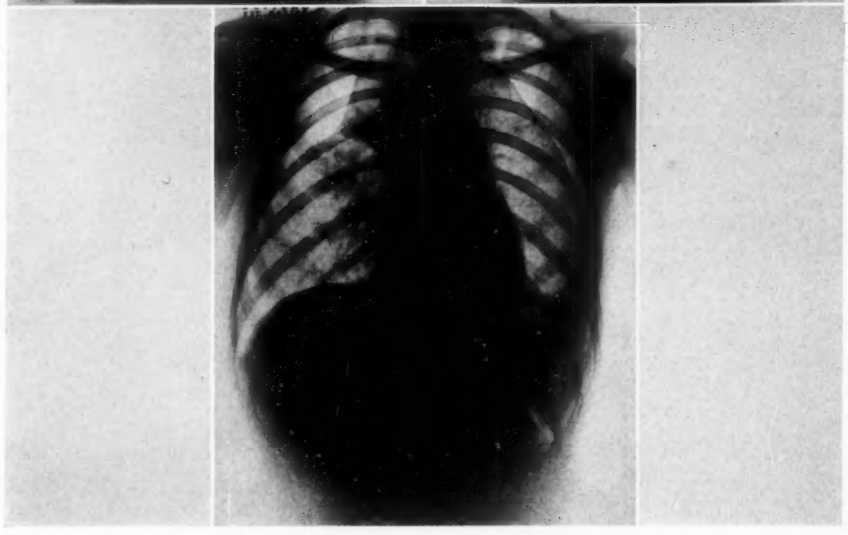

Fig. 3

from the pleural spaces in proportion than if just one lung is collapsed. This will naturally increase the frequency of refills.

The writer has under observation at present 12 cases of bilateral pneumothorax. These are being treated in the home and, although the management under home conditions is more difficult, they have responded more quickly and satisfactorily than cases of unilateral collapse. During the past year three have been lost by death. One had a spontaneous collapse into a superficial cavity which could not be corrected. Another

Case 2: Mrs. T. R. S., married, age 38. *Family history:* One sister died of tuberculosis; mother and two brothers have had tuberculosis. *Past history:* Influenza and pneumonia in 1921. No other illness or operation. Present history dates back to 1922 at which time a diagnosis of tuberculosis was made. The patient spent nine months in the State Sanatorium and almost regained her health, which she has done several times since, only to have a set back. She was first seen by the writer in consultation September, 1932, with the thought of instituting pneumothorax. Figure 4, made at that time, showed considerable fibrosis in both lungs with more in the right than in the left. It was not felt that pneumothorax was indicated. In addition to her lung involvement she was developing a tuberculous laryngitis. Watchful waiting, with absolute silence and complete bed-rest, was advised for the patient. She was seen again in February. At that time she had a cavity, 3 cm. in diameter, at the end of the second rib on the right side which could be diagnosed with the stethoscope as well as roentgen ray (figure 5). In addition there was considerable infiltration in the lower lobe of the left lung. Pneumothorax was advised for the right lung although it was felt very unlikely that a free pleural space could be found. Fortunately, there was no difficulty encountered in producing a selective collapse which is shown in figure 6, May, 1933. Adhesions noted in the right apex have proved an asset in that they permit a pneumothorax on both sides of the cavity which squeezes its walls together. This same film shows a cavity-formation in the lower lobe of the left lung. Pneumothorax at this time was advised for the left lung, and was started immediately. Figure 7, June, 1933, shows a selective collapse on both sides with the cavity closed on the right and the one on the left closing very nicely. It was remarkable how this controlled the clinical symptoms, particularly the cough. Her throat improved from the beginning, and has since healed completely. She is still under treatment and at the present time the right lung is being let out. Figure 8, January, 1934, shows the present condition of her lungs.

also had a spontaneous rupture, but there were no serious effects after the first twelve hours. She died eight months later, and postmortem examination showed a fibrocaseous tuberculosis with cavities in both lungs. There was no extrapulmonary infection. The third one died of extreme toxaemia and tuberculous laryngitis. These cases were unsuitable for any type of therapy but bilateral collapse seemed to offer the only hope. We are often forced to pursue a certain course of treatment when the outlook is grave and are many times surprised and gratified at the end-results.

Fig. 4

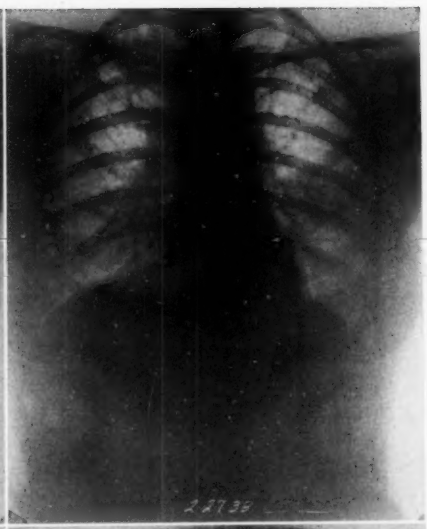

Fig. 5

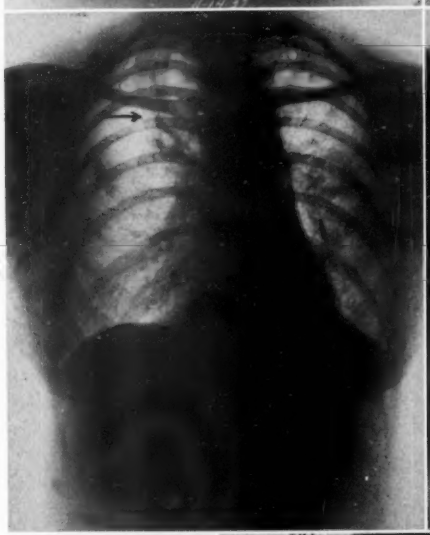

Fig. 6

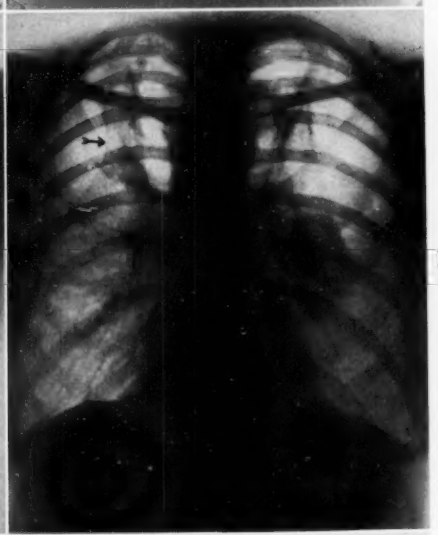

Fig. 7

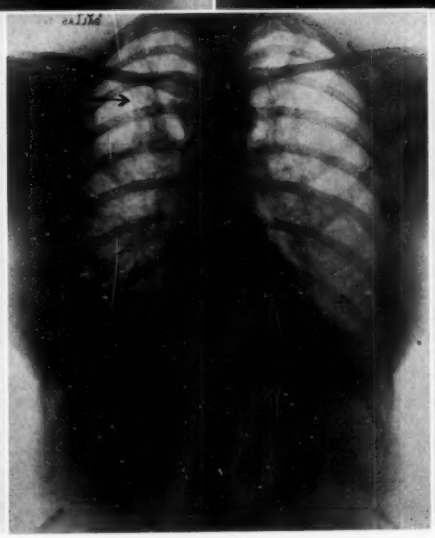

Fig. 8

Case 3: Miss R. D., age 23, student, came under my observation July, 1928. Family history was negative for tuberculosis. Her past history was essentially negative. She was taken ill in April, 1927, following an attack of influenza. Sputum was positive for tubercle bacillus. She had about a year of hospitalization following the diagnosis. The first examination, July, 1928, revealed a cavity in the lower lobe of the right lung about 5 cm. in diameter. The upper lobe of the left lung showed marked infiltration with threatening cavitation. She was running from one to two degrees of temperature. Pulse-rate was about 100; weight around 100 pounds. She was put on absolute bed-rest without showing any improvement. In fact, she began losing ground the latter part of August. A bilateral pneumothorax was advised but not accepted until October. Figure 9, made in October, showed no improvement in the right lung with several definite cavity-formations in the left and extension into the lower lobe. Pneumothorax was started in the left, first with 500 cc. of air at the first injection. The second day following, the right lung was started with 450 cc. of air at the initial injection. Both sides were kept at a 3 to 4 minus reading. Figure 10, February, 1929, shows a selective collapse on both sides. There was immediate improvement of symptoms. May, 1929, the patient developed an effusion in the right pleural space, and immediately the space began closing in spite of high positive-pressure readings which were maintained. Figure 11, August, 1929, shows the right pleural space completely closed with a medium compression on the left side. Although the right lung had reëxpanded the prognosis was more favorable than at any time in the past. A few days later she had an exacerbation of the tuberculous process in the lower lobe of the right lung. Her cough was uncontrollable. Fortunately, she still had good motion of the right diaphragm. A phrenicectomy was advised, which was done in September following. The operation was performed by Dr. Felix P. Miller, of El Paso. There was an immediate improvement. Figure 12, February, 1930, shows position of the diaphragm following phrenicectomy. Cough, temperature,—in fact, all symptoms,—immediately changed for the better. Weight increased to 139 pounds. Figure 13, May, 1934, shows the present condition of the lungs which has been unchanged for the past two and a half years. She has returned to the University to complete her education.

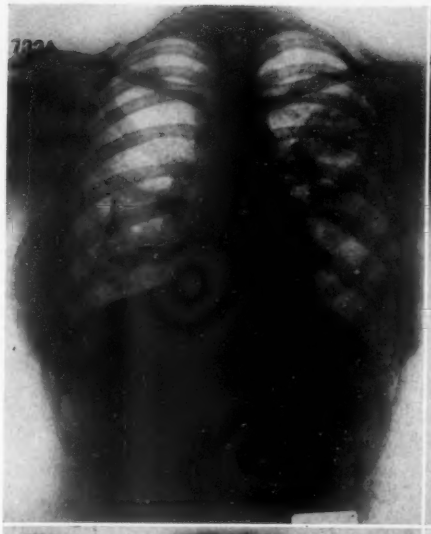

Fig 9

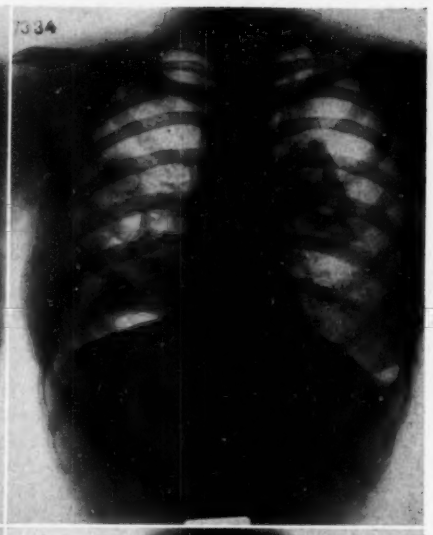

Fig. 10

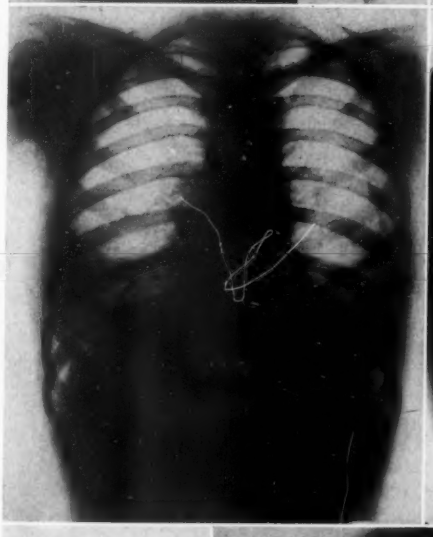

Fig. 11

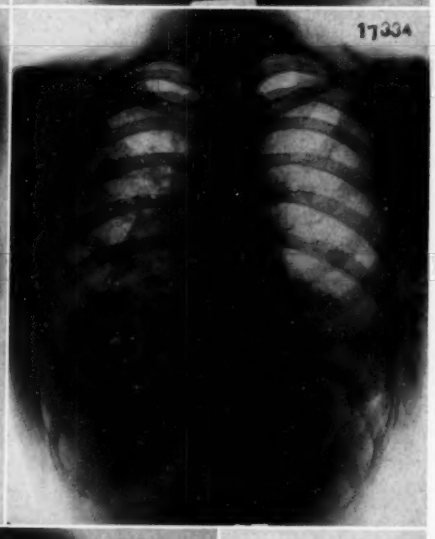

Fig. 12

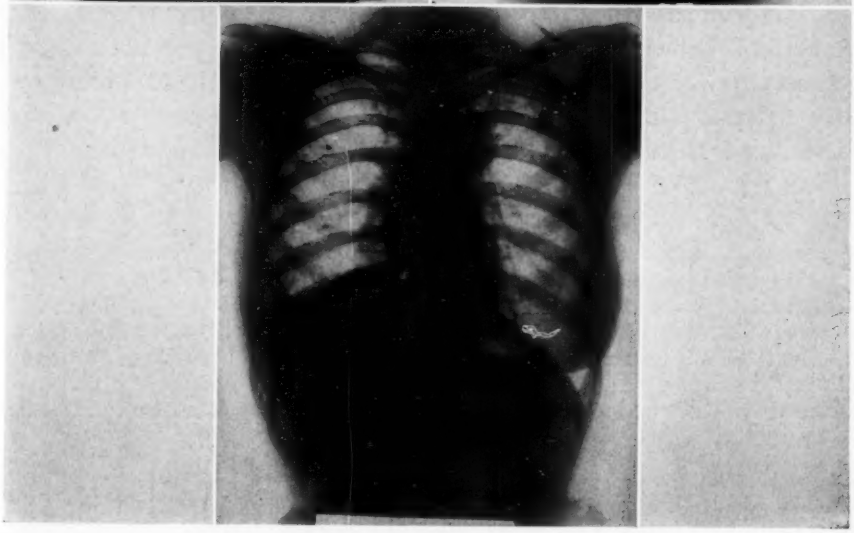

Fig. 13

These three cases illustrate practically every type of bilateral pneumothorax one wishes to follow. Sometimes, however, a patient is given unilateral pneumothorax and one side is completed before the opposite lung is started. This, of course, is unilateral pneumothorax in the same patient at different times. It is the writer's opinion that in doing bilateral pneumothorax it is preferable to start the more extensively involved lung first and secure a fairly good compression before starting the contralateral lung.

SUMMARY AND CONCLUSIONS

Of the 15 cases treated to the present time, three have died as reported above. Two are still doubtful. The other ten have been successful. One has had a phrenicectomy as a supplementary aid to pneumothorax.

Tice and Hruby reported 90 cases. Forty-three were successful; twelve had accessory measures such as phrenicectomy, oleothorax, and pneumolysis.

Pollock reported 59 cases of successful bilateral pneumothorax. All of these were unsuitable for unilateral pneumothorax alone.

Kaminsky reported two successful cases but they were complicated with effusion. This, no doubt, has been the experience of every one who has done pneumothorax either unilateral or bilateral. Effusion is rarely a serious complication.

The 15 cases reported above, together with the results reported by others interested in bilateral pneumothorax, lead the writer to conclude: First, that bilateral pneumothorax offers hope to otherwise hopelessly sick tuberculous patients with bilateral lesions. Second, the treatment should be instituted by some one who is familiar with pneumothorax in general and who has a knowledge of the physiology of the cardiorespiratory system. Third, the treatment should be started early before the formation of thick-walled cavities, fibroses, and pleuritic adhesions.

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BILATERAL SIMULTANEOUS ARTIFICIAL PNEUMOTHORAX IN THE TREATMENT OF PULMONARY TUBERCULOSIS

A Report of 36 Patients[1]

JOSEPH N. CORSELLO AND RALPH M. BRUCKHEIMER

The first successful demonstration of bilateral simultaneous artificial pneumothorax for the treatment of pulmonary tuberculosis was that of Ascoli in 1912. Until comparatively recent years, many considered the measure to be a therapeutic curiosity and looked upon it with disapproval. However, during the past decade or so, many favorable reports have appeared in the literature advocating this form of treatment as rational and of value in certain cases of advanced pulmonary tuberculosis. Among those who have made notable contributions to the American literature on the subject are W. R. Abbott, Barlow and Kramer, E. K. Geer, L. J. Moorman, Kinsella and Mattill, and Pollock and Marvin. All have employed the measure and without exception have been favorably impressed.

Bilateral simultaneous artificial pneumothorax was first employed here in 1927 by the late Dr. Robert F. Zimmerman. He was stimulated in this undertaking by the encouraging report of Kinsella and Mattill which was published that year. Before his untimely death in 1930, Dr. Zimmerman was successful in initiating the treatment in 16 patients. Since then we have added 20 cases, making a total of 36 being reported. None of these has had bilateral collapse started within the last one and one-half years, so that sufficient time has elapsed to warrant some fairly definite conclusions regarding the efficacy and value of the measure, at least as applying to the present group.

The primary purpose of this article is to report the authors' experience and results; so reference will have to be made elsewhere for information dealing with the academic and technical aspects of the procedure. These phases of the subject have been adequately discussed by previous writers and will not be dealt with at length here.

In general, the usually recognized principles have been observed in

[1] From the Newton Memorial Hospital, Cassadaga, New York.

administering and managing the treatment. Most of the patients comprising this series appeared to offer poor prospects for recovery if managed conservatively. There are included in the group a few border-line cases, that is, patients with extensive disease and whose resistance appeared to be at a low ebb. Some of these did not fare well, but a few obtained surprisingly good results. No effort was made to apply the treatment to obviously terminal cases, or to those who because of excessive disease were dyspnoeic even while at rest.

The series consists of 26 female and 10 male patients whose ages ranged from 16 to 43 years. The majority were in the 20 to 30 year group. Thirty-three were far advanced and three moderately advanced. Cavitation, demonstrable in the X-ray was present in 33 patients, 19 of whom had antrum formation bilaterally. The sputum was positive in 32, negative in 2, and was unobtainable in the remaining two.

According to the indications for the treatment, the cases may be classified into four groups:

Group 1: Twenty-five patients originally with unilateral pneumothorax who suffered extension of disease, already present in the contralateral lung. Simultaneous double collapse was given because it was felt that the primary pneumothorax could not be discontinued with safety, since the length of time in which it had been in effect was believed to be too short to assure thorough arrest of the disease.

Group 2: Three cases with unilateral pneumothorax originally. The reason for secondary pneumothorax was the appearance of a new focus in a previously uninvolved contralateral lung. As in group 1, it was felt that it would be unwise to abandon the primary collapse.

Group 3: Four cases with unilateral pneumothorax and moderately extensive disease in the contralateral lung. Secondary collapse instituted because these patients failed to show satisfactory clinical improvement after periods of observation ranging up to seven months. It was felt that if double pneumothorax were not administered, recovery might be delayed indefinitely if not actually jeopardized.

Group 4: Four cases with extensive bilateral, rather equally distributed disease, in which bilateral simultaneous artificial pneumothorax was the primary plan of treatment.

More or less serious extrapulmonary complications were present in 19 patients. There were 2 with compensated mitral regurgitation, one of which also had tuberculous laryngitis. Both were treated successfully and have arrested disease. Two others had ischiorectal abscess, one of which also had latent syphilis. The former of these is now well and has apparently arrested

disease. The other succumbed, but the extrapulmonary complications had no bearing on the outcome. In addition to the case mentioned above, there were 6 others with tuberculous laryngitis, of which 4 are living and have arrested or apparently arrested disease. The other 2 are dead, and in one of these the laryngeal complication contributed to the fatal outcome. Clinical tuberculous enteritis was present in 3 cases, of which one is now dead and 2 living and improved. The patient who expired was uncoöperative and abandoned the treatment after one month's trial. Four others had both tuberculous laryngitis and enteritis of which one is alive and has apparently arrested disease. The complications contributed to the fatal outcome in 2 of the 3 dead in this group. One patient had subacute nephritis, the result of an earlier attack of scarlet fever. This case also terminated fatally, the nephritis proving a major contributing factor. Tuberculous costitis was the complication in the last case in this group of patients. She has arrested her disease and is now well.

The most frequent complication encountered during the course of treatment was effusion into the pleural cavity. This occurred in 23 patients, or 63 per cent, of the series. In 18, the effusion was limited to one side. Aspiration for the relief of pressure symptoms produced by pleural exudation was resorted to in 6 patients. Premature loss of air-space following the development of effusion occurred in 5 cases, and diminution in the size of the pneumothorax cavity resulted once. Purulent exudates developed in 2 cases, one of which was successfully treated by means of gomenol oleothorax. One other case of empyema occurred which is not included above. This patient developed a bronchopleural fistula following closed intrapleural pneumolysis performed elsewhere. The empyema was a sequella of this fistula and could not be attributed to the treatment itself.

Spontaneous collapse occurred in 3 patients following initial attempts to introduce air. In one it occurred bilaterally, though on separate occasions. All 3 recovered, and it was possible to continue treatment thereafter. Repeated removal of air from the pleural cavity until the pleural opening closed was the chief treatment used for this complication.

Mild pleural shock with recovery occurred once.

No noteworthy untoward happenings occurred other than those recorded above. Cyanosis and difficult breathing were not encountered except in the instances of pleural effusion and perforation already mentioned. It is of interest to note that one patient, who ordinarily tolerated double collapse well, was discomforted at times during periods of very

warm humid weather and required temporary lessening in the degree of collapse.

Other therapeutic measures supplementary to bilateral pneumothorax were employed in 11 patients. Closed intrapleural pneumolysis was attempted in 8, one bilaterally. In 3 the operation was technically unsuccessful and no significant benefit ensued. One was technically successful, but derived no benefit because, even with the restraining adhesions severed, the rigid thick-walled cavities present failed to collapse. Another with a centrally placed cavity was also technically successful, but only partial cavity closure resulted, although the patient did show notable clinical improvement. In another case a pleurobronchial fistula developed shortly after the operation which indirectly led to death. The remaining 2 patients were operated on with benefit, being both technically and clinically successful.

Phrenicotomy was used once in a successful attempt to improve on an otherwise unsatisfactory collapse of one side. In another case, this procedure was employed with the same aim in view, but failed to accomplish its purpose. However, double pneumothorax was continued despite this until the disease in the effectively collapsed lung had become arrested. Thoracoplasty was then done on the opposite side. This patient has now been well for over 2 years. Without double pneumothorax it is extremely doubtful that she could have been brought to the point when a thoracoplasty could have been performed. Although the pneumothorax was unsatisfactory on one side, it did check the course of disease and permitted the patient's general condition to improve sufficiently to permit the operation without serious risk.

Gomenol-oleothorax was used in 3 patients. In one it was employed successfully to maintain pulmonary collapse which was endangered by obliterative pleuritis. In another it was used bilaterally with good effect to treat purulent effusions. Subsequently it was continued on one side as a maintenance oleothorax to prevent premature reëxpansion of the lung. The 3rd case in which oleothorax was employed was the one with the pleurobronchial fistula mentioned above. Both the fistula and the resultant empyema were successfully treated. Unfortunately, the contralateral pneumothorax had to be suspended for about 11 months during this period of oleothorax treatment and extension of disease occurred in the then uncollapsed lung. Secondary pneumothorax was reëstablished, but the resultant collapse was ineffective and the patient succumbed.

It should be pointed out that oleothorax has been used here only since

1930, and that intrapleural pneumolysis was not practised by us prior to the Spring of 1933. Patients requiring adhesion severance before the latter date had to go elsewhere for the operation. Only one had the financial means to do so. Consequently, a number of early cases in the series were obliged to do without these measures. It is probable that a few of those now dead might still be living if either pneumolysis or oleothorax had been available to them.

In considering the length of time that treatment should be given, we allowed ourselves to be guided almost entirely by our experience with simple unilateral pneumothorax. Double simultaneous pneumothorax, therefore, was administered just as long as we felt collapse was necessary and was producing benefit. There were, as has already been noted, 5 instances of premature loss of air-space, and consequently in these cases the duration of double collapse was involuntarily shortened. However, without making exceptions of these 5, the following figures relating to the time of actual simultaneous double pneumothorax have been compiled. As a matter of interest we have grouped the cases according to the present status of the patients.

Group 1: Total of 21 patients still alive. Shortest duration of double collapse 5 months, longest 52 months. Three with bilateral simultaneous pneumothorax of less than one year's duration as follows: 5, 8 and 10 months respectively. Nine between one and two years: 15, 15, 16, 17, 19, 21, 21, 22 and 22 months respectively. Seven between two and three years: 26, 27, 27, 27, 29, 33 and 36 months respectively. Two over three years: 44 and 52 months respectively. Included in this group are three patients still with bilateral collapse. Average duration of double pneumothorax for the group is 23.4 months.

Group 2: A total of 11 patients now dead, but who had been temporarily benefited. The shortest period for this group was 8 months; the longest 29 months. Five cases under one year as follows: 8, 8, 11, 11, and 11 months, respectively. Five between one and two years: 13, 16, 20, 21 and 22 months, respectively. One case of 29 months. Average for the group, 15.5 months. Included here is one patient who was uncoöperative and abandoned treatment after 13 months.

Group 3: A total of 4 patients now dead, who failed to benefit. The respective duration of bilateral collapse for this group was 2 weeks, 1, 1, and 5 months. Average for group, 1.9 months. Includes one uncoöperative patient who abandoned treatment after one month.

It should be noted that the above figures relate only to the length of time that bilateral simultaneous pneumothorax was actually in effect and not to

the primary and secondary collapses individually. Individual collapse usually was carried out for a longer period, inasmuch as primary pneumothorax antedated secondary collapse in all cases by as much as 7 months, and one side frequently was treated for some time after pneumothorax had been discontinued unilaterally.

PRESENT STATUS OF PATIENTS

Of the 21 patients still alive, 18 now have arrested or apparently arrested disease and seven have returned to work. It is expected that 9 others will, in time, be able to return to work. Two patients with arrested disease will probably never resume work: one because of a heart lesion, and the other, a war veteran, because of a psychoneurosis. Two of those living who have not yet achieved disease arrest are definitely improved (one is semiambulatory), but their prospects for recovery are not very bright, because of unsatisfactory collapse. The remaining patient of the 21 living, and heretofore unaccounted for, is again under rest treatment because of a recent reactivation of her disease. This patient appeared to be successfully treated and was clinically well for two years. An attack of influenza one year after treatment had been discontinued was followed by a flare-up of the lesion in her left lung. She has lately undergone a phrenicectomy, but appears to be a candidate for thoracoplasty, which will be done if the disease in the other lung remains inactive.

In three of the 15 dead the course of disease was unaffected despite good collapse. Two had caseopneumonic lesions, one of which also had subacute nephritis as mentioned previously. The third had a miliary-like pulmonary distribution of her disease.

Initial benefit was experienced by eleven of those now dead. Ten of these had unsatisfactory collapse of one or both lungs. Included here is the case with the pleurobronchial fistula that followed intrapleural pneumolysis as described above. Extrapulmonary complications contributed to the fatal outcome in 3 of these 11 cases.

Three cases are worthy of special comment. One concerns a male patient who was treated for $2\frac{1}{2}$ years, during which time he improved, became ambulatory and sputum-negative. Treatment was unwisely discontinued in the belief that his disease had become quiescent. However, soon afterward signs of activity became manifest and he failed rapidly. This case we feel demonstrates graphically the beneficial effect of the treatment. As long as double collapse was used, he progressed fairly

well, but as soon as it was discontinued he went downhill rapidly. The second concerns a patient who was treated for 13 months in the course of which she improved, became ambulatory and also sputum-negative. However, she tired of the treatment and returned to her home against advice. Subsequently we learned that she had been subjected to open drainage for a pleural effusion and had died shortly afterward. The third case is one in which treatment led to arrest of her disease. She was clinically well for three years, two of which followed termination of treatment. She then suffered an attack of influenza, followed by bilateral reactivation of her disease to which she succumbed in a few months.

The only other case that failed to coöperate well received treatment for one month. She failed to obtain any significant benefit.

It was mentioned previously that 11 cases received initial benefit. Three of these cases have been given in some detail above. This temporary benefit was characterized chiefly by varying degrees of initial constitutional improvement and lessening of symptoms. Practically all were more comfortable by virtue of the diminution of the severity of symptoms, and at least six experienced prolongation of life. Their demise when it came was in the majority of the instances more peaceful and comfortable and, because of this, we feel that the treatment was worth-while even though recovery did not occur.

Pneumothorax has been discontinued bilaterally in 13 of those still living. Included in this group is the patient with the thoracoplasty and also the case that has recently suffered reactivation of disease. Three patients still receive refills on both sides. Treatment has been discontinued unilaterally in 5 cases. One patient has pneumothorax on one side and maintenance oleothorax contralaterally. Another has maintenance oleothorax of the left side, but the right lung has been allowed to reëxpand.

Of those who have survived, 20 originally had cavitation in one or both lungs. Complete cavity-closure has occurred in 18 while in the other two it is incomplete.

It occurred to us that it might be of interest to note the fate of all those in the series who originally had pulmonary cavitation. It will be recalled that in 1928 Barnes reported his observations on 1,454 cavity cases that were treated without benefit of collapse. He found that 20 per cent were living and 80 per cent had died within one year after the appearance of cavitation; 15 per cent were living and 85 per cent had

died within three years and 90 per cent had died within five years. In our group of cases were 33 patients with cavitation. Three of these we feel should justly be removed from consideration. Two were uncoöperative and therefore did not give the treatment a fair trial. The third developed a pleuro-bronchial fistula following intrapleural pneumolysis and it was necessary to interrupt the treatment on the opposite side for 11 months while this complication was treated. Of the 30 worthy of consideration 20, or 67 per cent, were alive, and 33 per cent were dead two or more years after cavitation was first noted. Fourteen, or 47 per cent, have survived for four or more years and 11, or 37 per cent, between five and eight years. With the possible exception of three cases, we have every reason to believe that all of the above will continue to live for some years to come so that eventually the percentage of patients who will have survived for five years or more will be proportionately greater than at present.

If we were to include those patients now dead, but who survived at least one year and whom we believe experienced prolongation of life because of the treatment, the above figures would be even more impressive. In 6 such patients, life in our estimation was extended from one to three years. Adding these to the 20 above, we have 26 of the 30 cavity cases, or approximately 87 per cent, who survived at least one year after cavitation was first noted. It is realized that the statistical value of these results in such a relatively small number of cases cannot be very great. However, it must be granted that they do constitute additional proof of the superiority of collapse treatment for pulmonary tuberculosis over expectant treatment and also that they indicate to some extent the effectiveness of simultaneous bilateral artificial pneumothorax.

The effect of the treatment upon the sputum of these patients has been in keeping with the good results suggested above. A great majority experienced a lessening in the daily quantity of sputum and a corresponding diminution in coughing. Of 32 patients originally with positive sputum, 18 became consistently negative. In 7 others there was a definite lessening in the number of bacilli discharged in the sputum. The measure failed to alter significantly the nature and quantity of the sputum in only 7 cases of the series.

COMMENT

In the opinion of those who have used the measure, bilateral simultaneous artificial pneumothorax is a valuable and comparatively safe

form of treatment when properly employed. The end-results are dependent upon several factors, chief of which are (1) effectiveness of the collapse obtained, (2) proper selection of cases, (3) frequency of serious complications, (4) proper coöperation between patient and physician, and (5) the skill and diligence exercised in administering the treatment.

The most frequent cause of poor results is ineffective collapse due to restraining pleural adhesions. Judicious use of intrapleural pneumolysis and phrenicectomy in some instances will convert an unsatisfactory collapse into an effective one. As was previously mentioned, it is probable that the number of recoveries in this series would have been greater if intrapleural pneumolysis had been available to a few of the earlier cases in which inhibiting pleural adhesions were present. It is likely that with greater use of this valuable supplementary measure, ineffective collapse will not prove to be so great a factor as it has in the past and that the percentage of recoveries will be somewhat higher in the future.

In instances of unsatisfactory collapse of one side when adhesion severance is impossible, phrenicectomy will sometimes aid in converting the collapse into an effective one. However, even this will fail at times. In such cases, there are some who contend that bilateral pneumothorax should not be continued. We do not entirely concur in this opinion. Occasions will be met with when through continued use of bilateral collapse the patient can be prepared for thoracoplasty, as was done in one case of this series.

Because partial or selective collapse is the basis of bilateral simultaneous pneumothorax, the frequency of premature loss of air-space is rather high, especially following the appearance of pleural effusions. This distressing development can in some cases be successfully combated by early use of maintenance oleothorax. Our experience with this measure both for disinfectant and maintenance purposes has been quite satisfactory.

There appears to exist some difference of opinion regarding the choice of cases suitable for bilateral pneumothorax. The most favorable subjects are those with disease limited to the upper lobes of both lungs. Some authorities contend that the treatment should be limited to this type of patient and that it should not be employed when the disease is extensive and the patient's resistance appears to be poor. In our opinion such a contention is unwarranted, inasmuch as there is no method known whereby a given patient's resistance can be accurately measured and also because the response to treatment cannot be predicted in advance.

Some of the most unlikely-looking cases will at times give surprisingly good results. Consequently, if one were to observe strict standards in the choice of cases he is very apt to allow some, who might have been saved, to die prematurely. Following a liberal interpretation of the indications for the treatment will of course result in the inclusion of some cases with whom no striking success will be obtained. However, even an occasional good result would justify the use of the measure under those circumstances, for, if left alone, these border-line cases usually prove fatal in a short time.

It is hoped that our attitude in this respect will not be misinterpreted. We do not advocate that all cases of advanced tuberculosis be subjected to bilateral pneumothorax irrespective of the patient's condition. There are patients who respond satisfactorily to simple rest treatment, when the disease is not rapidly progressive. This type can usually be recognized and, as a rule, does not require any form of collapse treatment. On the other hand, the measure should not be considered an agency of last resort, although in most instances in which it is employed it is actually life-saving. Patients, who are obviously terminal or who are dyspnoeic even while at rest because of extensive disease, should not be subjected to the treatment.

Those with caseopneumonic lesions have in our experience responded poorest to the treatment. There were 8 such cases, of which 7 have died and the other has improved. Two of the dead did not respond at all even though the collapse obtained was good. Temporary improvement, however, did occur in the other five.

The question of complications, both those occurring during the course of treatment and those which are extrapulmonary, is worthy of additional comment. Those which are met with in the course of treatment are the same as occur in unilateral pneumothorax. The most spectacular and distressing complication, of course, is that of a ruptured lung. However, the possible occurrence of such complications should be no deterrent to administering the treatment. While they do occur with more frequency than in unilateral pneumothorax, this increased incidence is not great enough to contraindicate use of the measure.

Coulaud, among others, advises that patients with clinical tuberculous enteritis should not be treated by this method. He had some rather disappointing experiences with patients suffering from this extrapulmonary complication. However, we cannot wholly subscribe to this view. An involvement such as this undoubtedly adds to the hazards

of the case, but recovery is possible in certain instances if satisfactory pulmonary collapse is obtained.

In reviewing our cases who had intestinal tuberculosis and in whom the result was unsuccessful, we find that without exception all of them had unsatisfactory collapse of one or both sides. It is conceivable that with effective pneumothorax the result might have been different in these instances. One of our patients who was gravely ill, and who had a severe intestinal involvement, made a gratifying recovery after both lungs were effectively collapsed.

SUMMARY

1. Thirty-six cases of advanced pulmonary tuberculosis were treated by bilateral simultaneous artificial pneumothorax. In none was treatment started within the last 1½ years.

2. Twenty-one are still alive, 18 of whom have arrested or apparently arrested disease and two are improved. One has suffered a recent reactivation of disease after being well for two years, one year of which was after termination of treatment.

3. Fifteen are dead of which 3 received no benefit whatever. One other was uncoöperative and was unbenefited by one month's treatment. Eleven cases received temporary benefit. One of these discontinued treatment against advice, after 13 months, when she appeared to be on the road to recovery, and died at home following injudicious open drainage for a pleural effusion. Another obtained arrest of disease, and was well for three years; 2 of which were following termination of treatment. She succumbed following a later reactivation of disease induced by an attack of influenza.

4. Seven of those now living have returned to work. Nine others should be able to work in the near future.

5. The most frequent complication encountered during the course of treatment was pleural effusion, which occurred in 23, or 63 per cent, of the group. Spontaneous collapse occurred in 3, or 8 per cent, of the cases.

6. The chief cause of failure of the treatment was ineffective collapse due to pleural adhesions. Intrapleural pneumolysis and phrenicectomy aided in converting 3 of these into effective collapses.

7. Cases with caseopneumonic lesions responded poorest as a group.

CONCLUSION

Bilateral simultaneous artificial pneumothorax is a valuable, effective and comparatively safe form of treatment for certain cases of advanced pulmonary tuberculosis that appear to offer poor prospects for recovery if managed conservatively.

We gratefully acknowledge the kind encouragement and coöperation of Dr. Walter L. Rathbun, Superintendent of the Newton Memorial Hospital, which in a large measure made this work possible, and also for his assistance in preparing this paper. We are also indebted to Dr. Harry A. Bray and Dr. Mac McConkey of Ray Brook for their constructive criticisms.

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Cases 1 & 2

Case 1: Illustrates a case of extensive pulmonary tuberculosis involving both lungs in which bilateral pneumothorax was not indicated and hence was not employed. D. B. White, female, married, 30 years old, admitted August 14, 1934, with illness of 2 years' duration. Upon admission, had slight productive cough, afternoon fever to 99°F. and occasional right chest pain. There had been no recent loss of weight but the patient was thin. Her sputum contained tubercle bacilli (Gaffky III).

Her admission X-ray (figure 1) showed extensive mottling in both lungs, heaviest above 5th anterior ribs, with multiple cavities behind right 1st and 3rd interspaces and left 2nd rib. Suggestive annular-shadow formation was noted behind right 3rd rib. Both upper lobes presented a honey-combed appearance.

On ordinary sanatorium regimen the patient improved steadily. Her symptoms disappeared in 2 months and her sputum was repeatedly negative after December, 1934. To date, 11 months after admission, she has gained 20 lbs., and is now on limited exercise. The X-ray appearance of her disease has shown marked improvement as shown in figure 2 taken July 10, 1935.

Case 2: R. M. White female, married, 24 years old, admitted, August 5, 1932, with illness of 3 months' duration. There was severe cough with moderate amount of expectoration, weakness, loss of 15 lbs., night-sweats, and fever ranging to 101°F. The sputum contained tubercle bacilli.

Her admission X-ray of August 6, 1932 (figure 3), showed extensive mottling in both lungs, suggestively miliary-like, heaviest in the left apex and 1st interspace. A small cavity was noted behind the left 1st interspace.

After a brief period of observation, bilateral pneumothorax was decided upon because the patient was rapidly failing. Left side was started on August 18, 1932 and the right on October 3, 1932. The resulting collapses appeared to be fairly good, only the left apex remaining unsatisfactorily compressed due to pleural adhesions (figure 4). However, her course continued downhill without interruption, and she expired on October 28, 1932.

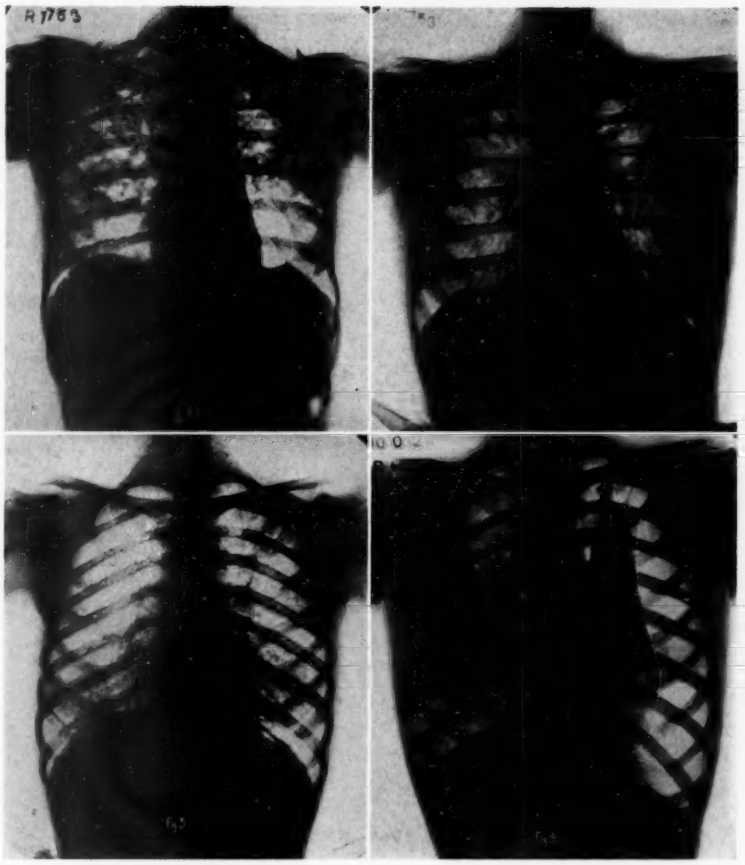

PLATE 1

Case 3

Case 3: A. C. White female, married, 27 years old, admitted, April 24, 1927, with illness of 6 months' duration. Upon admission the following symptoms were present: Moderate cough, moderate to scanty expectoration of positive sputum, weakness, loss of weight, sharp pain in base of left chest, hoarseness and laryngeal pain, and afternoon fever ranging to 100°F.

Her admission X-ray showed coarse mottling in the left upper lobe above the 4th rib, with cavitation in apex and behind the 2nd rib; scattered mottling in the right upper lobe above the 4th interspace.

Left pneumothorax was advised but rejected. Two weeks later disease extension occurred and symptoms increased with fever to 103°F. The X-ray prior to pneumothorax treatment is shown in figure 5. The left lung selectively collapsed starting on May 5, 1927, but subsequent improvement was not marked. Temperature elevation to 101° persisted. The disease in the right lung slowly progressed and enlarging cavitation occurred (figure 6). In February, 1928, the patient raised blood-streaked sputum, and it was decided to collapse the right lung also, with the result shown in figure 7.

Her course following this was steadily favorable. Her sputum became negative in October, 1928. Before her hospital stay terminated on January 22, 1929, she had gained about 50 lbs. Following her discharge she returned for refills, and reported that she was performing light tasks in her home. Pneumothorax on the right side was discontinued on February 9, 1931, and on the left side, November 24, 1931, with the result as shown in figure 8. She has remained well for over 6 years.

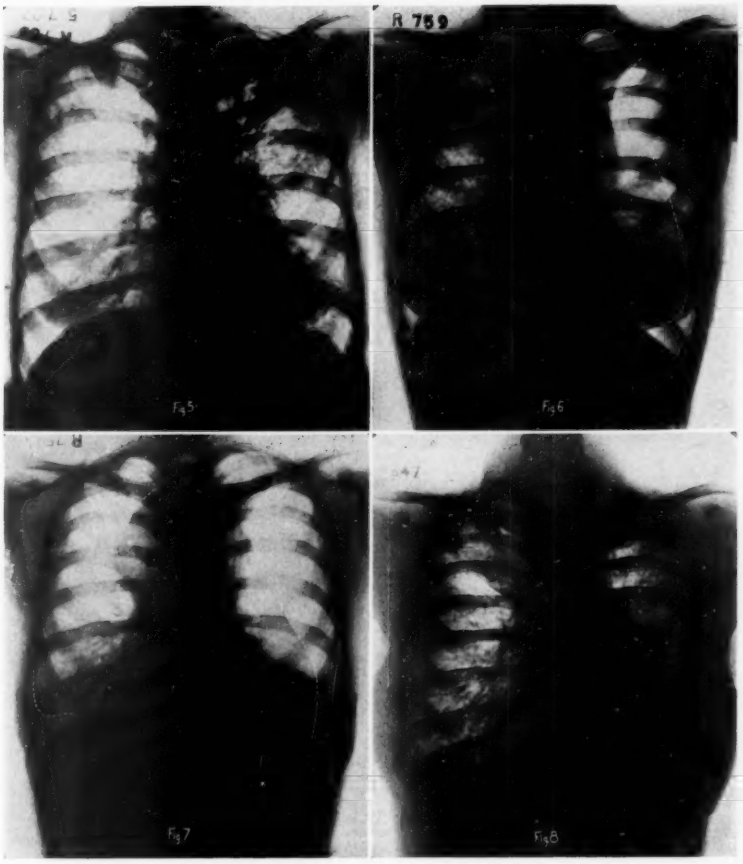

PLATE 2

Case 4

Case 4: Presented to illustrate the use of maintenance oleothorax to supplement bilateral pneumothorax. A. E. White, female, 33 years old, single, admitted May 9, 1931, with moderately advanced disease of 2 years' duration. Chief symptoms were moderate cough and expectoration, loss of 5 lbs., weakness, hoarseness, and fever ranging up to 102°F. The sputum contained tubercle bacilli. Laryngeal tuberculosis was also found.

Her X-ray (figure 9) showed dense infiltration on the left side, above the 3rd interspace, with softening and rarefaction behind the 1st interspace. An annular shadow noted also near left hilum. Mottling in right lung above the 3rd rib with an annular shadow behind the 2nd rib.

Left artificial pneumothorax started on July 6, 1931, after one month of bed-rest had failed to produce satisfactory improvement. Apical adhesions inhibited the collapse.

Definite symptomatic and constitutional improvement followed, but in October the X-ray revealed increased cavitation in right upper lobe (figure 10). Right pneumothorax started November 2, 1931, and further improvement resulted. Collapse of the left lung subsequently became more effective (figure 11). In December her sputum became negative for tubercle bacilli.

She was discharged from the hospital on December 22, 1932, after which she returned periodically for refills. In the Spring of 1933, the left lung began to reëxpand and, rather than risk losing the collapse, maintenance oleothorax was induced, with the result as shown in figure 12. Pneumothorax is still being continued on the right side. The patient resumed her occupation of dressmaker $1\frac{1}{2}$ years ago and has worked steadily despite the bilateral collapse.

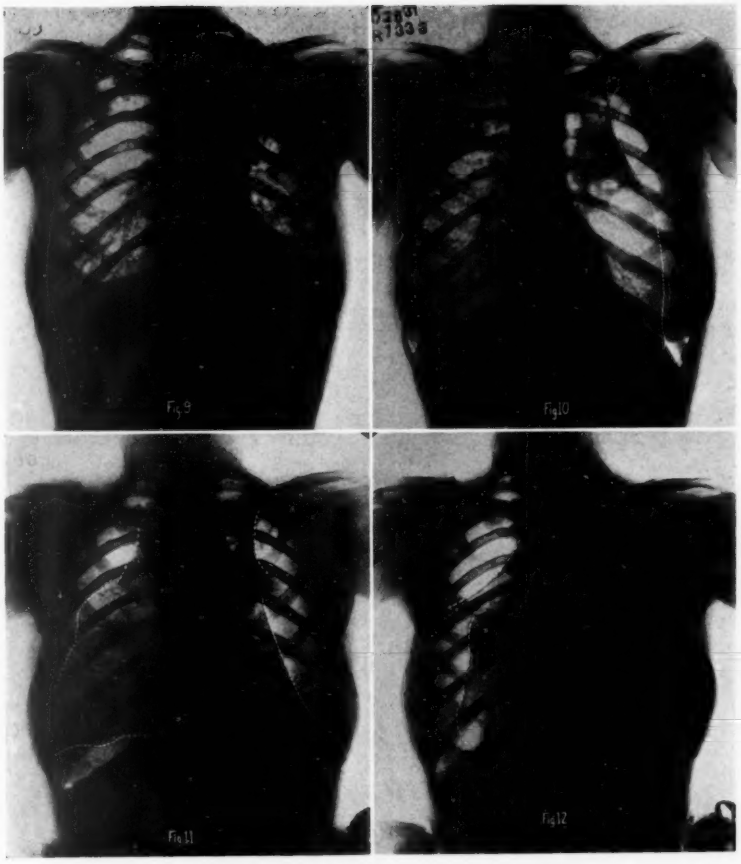

PLATE 3

Case 5

Case 5: H. H. White female, married, 33 years old, admitted on July 10, 1931, with far-advanced pulmonary tuberculosis of three years' duration. Upon admission she complained only of slight nonproductive cough and loss of strength. The temperature was normal.

Her X-ray showed linear-mottled infiltration in right lung, most marked in upper lobe, with antrum formation behind the 1st interspace; fibrosis in the left upper lobe and marked calcification in the left hilum. The right diaphragm was elevated and the heart and mediastinum were displaced to the right.

One week after admission, the patient became acutely ill, with fever to 103°F., and increased cough moderately productive of bacillary sputum. A portable X-ray (figure 13) showed new disease in the left lung. Left artificial pneumothorax was started on August 3, 1931, with a good collapse resulting. However, subsequent improvement was unsatisfactory. Right pneumothorax was started on November 9, 1931. Pleural adhesions prevented effective collapse of upper lobe. However, the patient's symptoms lessened considerably, and she improved generally although her sputum remained positive.

On May 15, 1932, right phrenicectomy was performed in the hope that collapse would be improved, but, although a good diaphragmatic rise was obtained, no benefit resulted (figure 14).

In June, 1932, the right lung began to reëxpand rapidly, due to adhesive pleuritis and there was a return of symptoms, with increased cough and expectoration and fever of 102°F. Treatments were discontinued on both sides in August, and right extrapleural thoracoplasty was performed in September, 1932. Ten ribs were sectioned in two stages. Recovery was rapid. Practically all symptoms had disappeared after one month and the sputum became negative. She left the hospital on August 20, 1933, and has remained well since, and has been able to perform light tasks in her home. Figure 15 shows the condition of her lungs as of July 2, 1935.

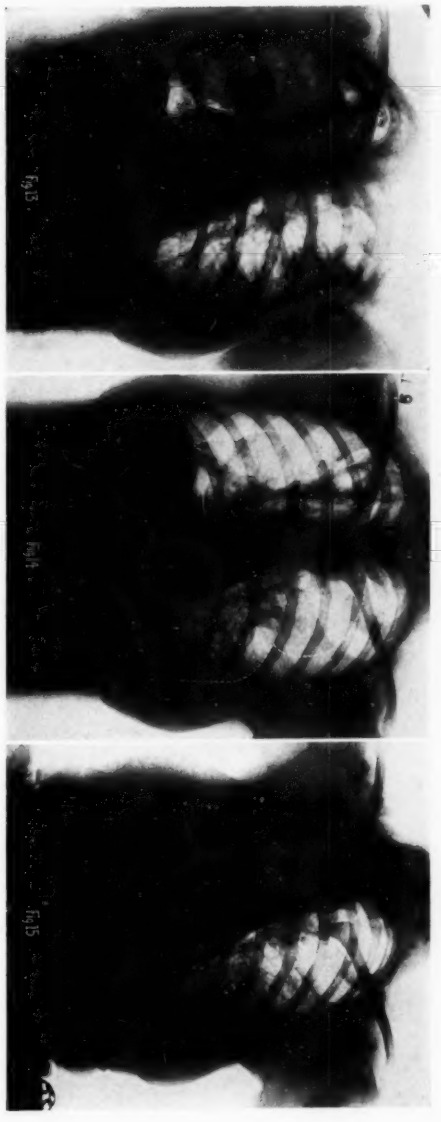

PLATE 4

EFFUSION FOLLOWING ARTIFICIAL PNEUMOTHORAX

A Review of 150 Cases at Essex Sanitarium, Middleton, Massachusetts

J. I. WEISMAN[1]

In the course of treatment of pulmonary tuberculosis by artificial pneumothorax, pleural effusion is by far the most common and often a very serious complication. The very small effusions which are usually transitory and only discovered during routine fluoroscopic or X-ray examination are rarely of any consequence. In this review we shall limit ourselves to those cases which show appreciable fluid, the level being above the diaphragm and producing clinical symptoms, or at least affecting in some way the ordinary course of the treatment, that is, the type usually referred to as the "fluid case."

This study comprises a series of 150 consecutive and unselected cases. Of this number, there was a minimal amount of fluid in 54 cases, or 36 per cent; appreciable fluid in 72 cases, or 48 per cent; a total of 126 cases, or 84 per cent, showing an effusion varying from a trace to one which filled the entire hemithorax. Some writers say that effusion occurs in every case, but is not discovered because of resorption between examinations. This study is limited to the 72 cases which showed appreciable fluid.

TABLE 1

AUTHOR	NUMBER OF CASES	EFFUSION	CLEAR	PURULENT
		per cent	*per cent*	*per cent*
Hayes (9)	151	82	61	21
Burrell (3)	309	41.4	29.8	11.6
Boonshaft (2)		47		
Stobie (14)		50		
Nalbant (14)		53.5		
Dumarest and Brette (14)	265	86	70	16
Matson, Matson and Bisaillon (12)	420	29.3	17.1	12.2
McKinney (14)				5
Naveau (14)	534			20
Peters and Wooley (15)	142			6
Essex Sanatorium	150	84		

[1] Springfield Hospital, Springfield, Massachusetts.

Age: Age as a factor is not of any special significance since the age incidence of effusion is similar to that of artificial pneumothorax *per se.* Table 2 shows the distribution of cases both by age and by sex. Over 50 per cent are to be found in the age-group, 20–30.

Side: The side most often affected in our series was the right,—45 on the right side and 27 on the left: this corresponds to the proportion found in cases of artificial pneumothorax as a whole.

As to sex, there were 28 males, or 52 per cent of 54 male cases, and 44 females, or 46 per cent of 96 female cases, so that sex does not play a significant rôle in the occurrence of pleural effusion.

Stage of disease: Forty-nine cases, or 67 per cent, were classified as far advanced; of these 17 were male and 32 female.

Twenty-three, or 33 per cent, were moderately advanced,—11 male and 12 female. Stage of disease also is not a factor in the occurrence of pleural effusion, since about 65 per cent of our artificial-pneumothorax cases are in the far-advanced group.

TABLE 2

AGE	MALE	FEMALE	TOTAL
15–20	3	5	8
20–25	6	13	19
25–30	6	12	18
30–35	3	5	8
35–40	4	3	7
40–45	4	3	7
45–50	2	3	5
Total.....	28	44	72

Duration of disease before artificial pneumothorax was instituted: It was thought interesting to see how soon in the course of the disease artificial pneumothorax was induced. It is well known that in years past the "dangerous" collapse method was not resorted to until bed-rest had been given a long trial, one or more years. Nowadays we are instituting the deflation procedure very early in the disease in chosen cases; sometimes, almost as soon as the case is discovered. Since this series takes in several years, it is to be expected that cases will be found at both chronological extremes. Thus we find that the duration varies from three months to nine years. The average for males is thirteen months,—the

mean, eight months; for females the average is eighteen months,—the mean, ten months; for the group as a whole, the average is sixteen months, —the mean, nine months.

TABLE 3

Duration of disease before artificial pneumothorax

Male:	Average 13 months; mean, 8 months
Female:	Average 18 months; mean, 10 months
For whole:	Average 16 months; mean, 9 months
Range from 3 months to 9 years	

Duration of artificial pneumothorax when effusion appeared: Effusion may appear at any time in the course of treatment and also when the collapse has been discontinued. Fifty-four cases, or 75 per cent, make their appearance within the first six months of treatment; 66, or 91 per cent, appear in the first year. Table 4 shows the number of cases developing at different stages of treatment. The third month seems to be a peculiarly apt one in which to develop effusion and directs our attention toward the utilization, at that time, of any prophylactic measures available.

TABLE 4

Duration of artificial pneumothorax when fluid appeared

1st month, 4	
2nd month, 8	
3rd month, 16	
4th month, 6	
5th month, 9	*Total*
6th month, 11	54–75%
6–12 months, 12	66–91%
12–18 months, 3	
18–24 months, 2	
24 months, 1	
Total, 72	

Amount of fluid: As stated earlier in this paper, the amount of fluid may vary from just a negligible trace discovered routinely by roentgenological examination to a massive effusion with symptoms of discomfort, dyspnoea and a sense of fulness produced by the displacement of the mediastinal organs. In table 5 will be seen the distribution of cases according to the highest level attained by the effusion before aspiration was resorted to. The level is determined anteriorly by the costochondral

junction. Forty-six cases, or 62 per cent, are between the 4th and 6th ribs.

TABLE 5
Amount of fluid

1st rib, 6	
2nd rib, 3	
3rd rib, 7	
4th rib, 16	62 per cent
5th rib, 15	
6th rib, 15	
7th rib, 8	
8th rib, 2	
Total, 72	

It is often easy to forecast the development of fluid several days before it actually makes its appearance. There is sometimes a sequence of symptoms which calls our attention to this possibility. During a refill, the patient may complain of pain in the collapsed side where previously the procedure has been relatively painless: this sign frequently precedes the effusion (11). Or, the onset may closely simulate that of ordinary "grippe," with general malaise, a sharp rise in temperature, aches and pains, anorexia, nausea and vomiting, pleuritic pain, or, with a large amount of fluid, dyspnoea, a feeling of tightness, and palpitation. Fluoroscopy may at this time reveal the fluid, or it may fail to make its appearance for two or three days; some patients will have symptoms of pleurisy without any fever, others will have no symptoms whatever, and the effusion will be discovered only when the patient complains of a splashing sensation within his chest with change of position. Or, again, it may be suspected when, during the routine refill, the operator finds that it takes a much smaller volume of air to attain the usual intrapleural pressure than at previous refills; for instance, where 450 cc. were necessary at seven-day intervals to leave the pressure at zero, it now takes but 100 cc. In our series 57 cases, or 79 per cent, had a symptomatic onset, while 15, or 21 per cent, were asymptomatic.

The fever when present varies a great deal in duration depending upon the type and amount of effusion. Some will have a sudden and unexplained rise of temperature to 100°–102°*F.* for only one day, and the X-ray will then reveal fluid. Usually, the duration is about two weeks, but there have been some cases which will run a persistent septic temperature for six or more months. These longer febrile periods are just

as common with the clear as with the purulent effusions. The average for the entire series has been nineteen days.

The average number of refills for the series before fluid appeared was 18; the average volume 350 cc.; the average mean pressure, — 2,—so that no undue tension or stress was imposed upon the collapsed lung.

As to type of fluid, our figures are not truly indicative since previously fluid cases were seldom aspirated. More recently, however, we have aspirated all cases with inordinate amounts of fluid or those who have exhibited pressure symptoms. Thus, of 72 cases, 39 were not determined as to type. Of those aspirated, 23 were clear and 10 purulent. Of the originally clear fluids, 7 later became purulent.

We have endeavored to present unbiased facts and figures with as little critical comment as possible. Before going on to the discussion of complications, treatment, prognosis and end-results, we should like to discuss the pathology and aetiology as far as such factors are known.

Many authors have stated that the effusion following pneumothorax is never a transudate but a true tuberculous exudate (4) (5) (20). A fresh effusion is usually clear, and contains a few red blood cells, endothelials and lymphocytes; and no secondary organisms unless introduced. Rarely tubercle bacilli are seen, although in older effusions they are much more common but can only be proved to be present by smear, culture and guinea-pig inoculation; older fluids become thick and turbid, the fibrin content, albumen, specific gravity increase, also the cells and tubercle bacilli; some have reported a high eosinophile count (13). We have not been as successful as others in proving the fluid to be tuberculous although we have used guinea-pig inoculation extensively; a smear is seldom of help. Hayes (9) reports 10, of 23 cases, with tubercle bacilli in the fluid. Pinner and Moerke (16), 12 out of 15. In 25 specimens examined, we have found 4 positive by smear and six by guinea pig; only one specimen showed secondary organisms (a case undoubtedly with pleurobronchial fistula).

Pinner and Moerke (16) in a very complete study of pleural effusion have concluded as follows: There is normally permeability of the pleura in either direction and it eliminates most products very easily; the persistence of effusions following pneumothorax is not due to any chemical changes but to a change in the permeability of the membrane. Thus the pleura becomes thickened and impermeable, and there is a failure in absorption of the tuberculous exudate.

There are a great many theories and explanations offered to account

for the occurrence of these effusions. Their number and variety show at once either that we are quite in the dark concerning the true cause or that there is more than one factor involved, or again that there are different types of effusions resulting from different causes.

One of the earliest explanations offered is the difference in temperature between that of the air injected and that of the pleural space. Berlin went so far as to devise a coil-heater to preheat the air before it is injected. Andreani has made the statement that effusions occur more frequently in winter than in summer. Table 6 lists the distribution of cases in our series according to season, showing very little variation in incidence. Another reason commonly offered is that of too rapid induction of collapse with use of excessive pressures and volumes of air. In our work, we have endeavored to avoid such faults, and yet our incidence of effusion has not been cut down. Some have regarded air as an irritant, but this does not explain the difference between the occurrence of minimal and large amounts. The introduction of minute particles of tissue or bits of foreign matter has been offered as a plausible reason but is somehow not wholly adequate, although Nalbant looks with great favor upon this theory (14).

TABLE 6

SEASON	NUMBER
Spring	19
Summer	17
Fall	20
Winter	16

Joannides (11) divides his causes into both endogenous and exogenous factors, regarding the latter as of great importance and urging the careful use of aseptic technique. As to endogenous causes, he brings out a most important factor, namely, rupture of existing adhesions with infection of the pleura. The injured pleura comes in contact with contaminated air and this produces an exudate. He compares the ruptured lung very aptly to a peritonitis produced by a ruptured abdominal viscus. From a microphotographic study of the pleura, he points out the existence of tubercles on the surface of the lung breaking off and infecting the pluera.

Stivers (21), in his beautiful pictorial work on pneumolysis, has pointed to the existence of exudative plaques on the surface of the lung usually close to adhesions, and he suggests that perhaps this may be a factor.

In the author's mind, the rôle of the adhesion has for a long time been very prominent. It seems very logical that tension on and finally rupture of an adhesion should produce an irritation and subsequently an effusion, especially when infection is thereby introduced. Thus we have effusion occurring early in the treatment when adhesions are most likely to be present (14). Effusion usually follows the intentional severance of a pleural band as in pneumolysis. Effusion has developed thus far in 14 of 16 cases allowed to reëxpand following completion of pneumothorax treatment, pointing to rupture of tiny adhesions formed during the long period of compression. In a careful review of the X-rays of our series, the author and other members of the staff were struck by the coincidence of pleuropulmonary symphyses or adhesions in 100 per cent of cases developing effusion. Of cases which did not develop fluid, either a complete "jacket" collapse was obtained or the adhesion when present was so thick that it could hardly be affected by the collapse. In five of our cases, it could definitely be shown that effusion occurred immediately after rupture of an adhesion. Ford (6) says that this will occur if the rupture is at the site of a tubercle.

As to the significance of fluid, is it a deleterious factor in the treatment of tuberculosis by artificial pneumothorax? This is best decided when we review the complications which may occur with effusion. The most common and easily treated is a shift of the mediastinal organs to the contralateral side with resulting dyspnoea, palpitation, cyanosis, and sometimes extreme respiratory distress. This shift is seldom serious, and may be treated by simple aspiration. Another complication is that the effusion, clear at first, may become purulent, and develop into a true chronic tuberculous empyema, difficult to clear up entirely and very likely to complicate matters further by rupturing into a bronchus, thus forming a pleurobronchial fistula, with secondary infection of the purulent effusion; or by rupturing through the chest-wall, thereby forming either a chest-wall abscess or a chronic draining sinus; also from the long-standing purulent infection amyloid disease may develop (14).

A most important complication, and one frequently responsible for the failure of pneumothorax, is the adhesive pleuritis which may ensue. It appears that the fluid formed is not a good compressing agent, since it tends to produce pleuropulmonary symphysis with resulting expansion of lung beneath the fluid-level. This creeping-out process of the lung usually takes place from the base upward (2). The end-result is that the pleural space becomes obliterated; diseased lung is reëxpanded; and

sputum, previously negative, may become positive once more. Finally, with the diseased area wide open, there is a greater chance of extension of disease to the contralateral or better lung or to other parts of the body. Such obliteration is the most common factor responsible for the discontinuation of what otherwise might have been a successful artificial pneumothorax (14). Fluid also is responsible for increase in fibrosis of lung and pleura thereby interfering with proper expansion of the lung and also resulting in a marked displacement of trachea and mediastinum to the ipsolateral side.

There is, however, a brighter side to this problem of effusions. We have seen in many cases that the additional compression produced by the fluid has resulted in clinical improvements and a more complete collapse. Thus in 17 cases of our series sputum was rendered bacilli-free shortly after the appearance of an effusion. Duffy of England makes the statement that cases with effusion do better than those with dry pleurae. This does not coincide with our experience.

Before passing on to the treatment of fluids, we must say just a few words, perhaps futile, regarding prophylaxis. Of course, the usual precautions should be taken, namely, sterile operative procedure throughout. The field of operation should be aseptic, the instruments well sterilized, the operator should wear sterile gloves; the fluid cases should be treated as infected, and reserved for the end of the day's work, doing the clean cases first and the others afterwards. One should not use too great volumes or too great pressure of air; the use of too great positive pressures to stretch existing adhesions is not advisable. The temperature of the room should be warm to prevent chilling of the patient. "Epidemic" colds should be isolated. These are all general and obvious measures.

Guth (8) and Boonshaft (2) have used intravenous calcium chloride as a preventive measure. In the latter's series of 15 cases so treated only one developed an effusion. Calcium chloride is also said to be of use in the treatment of effusions to hasten their resorption; the suggested dose is 4–5 gr. given orally and supplemented with digitalis. This procedure should be tried out more widely for a more exact estimate of its worth.

The subject of treatment should be divided into two separate parts; the clear and the purulent effusions. In both, the simplest method is that of *laissez faire*, leaving them alone to absorb spontaneously; this, of course, where the effusion is not too great. However, most writers agree that nontreatment is too conservative, since clear fluid often

becomes purulent and tends to obliterate the pleural cavity. Duboff reported 20 untreated cases; six ruptured through chest-wall, four into a bronchus and the rest developed amyloid disease. There are definite indications for treatment, as outlined by Chandler, as follows:

1: Prolonged pyrexia
2: Large amount of fluid
3: Excessive shift of mediastinum
4: Protraction of effusion
5: Threatened obliterating pleuritis
6: Empyema

Nalbant (14) says that, when the effusion lasts more than four months, aspirate frequently and replace with air to the desired pressure. This is best done according to the Gravesen (7) method in which aspiration and replacement are carried on simultaneously, thereby preventing the troublesome and sometimes painful mediastinal shift. The latter method has been found by the writer to be very efficacious.

Very many chemical solutions have been instilled into the chest in an effort to sterilize the pleural cavity. The results have been so variable that no conclusion can be derived from existing reports. Formalin in glycerine, once a favorite agent, has been found to be much too irritating and presents also the danger of producing pleurobronchial fistulae. Methylene-blue, gentian-violet, mercurochrome, have all been used but with no consistency of encouraging results. I believe the only effect of these dyes which is of value is the sterilization of the needle-track in the course of aspiration. Recently, gomenol in olive oil has been used as a replacement medium for the aspirated fluid; its effect is said to be both antiseptic and lytic (18) (21). We have used it in very few cases, but the results were not at all encouraging. Metaphen-in-oil likewise is said to be effective, but has not been sufficiently widely used to judge its efficacy. Rudman and Ellison (18) make the following claims for it: (a) it is well tolerated, (b) does not thicken pleura, (c) prevents adhesive pleuritis (d), is highly bactericidal, and (e) liquefies cellular elements.

We, therefore, return to aspiration. This seems to be the best method of treatment but only if done early and if the collapse is maintained by replacement with air. Of our 72 cases, 32 were aspirated at irregular intervals. The duration of persistence of the effusion was ten months in the clear cases and twenty-two months in the purulent cases. However, the average for the untreated cases was only nine months, but it must be pointed out that a great many of the latter were mild and showed a tendency toward spontaneous resorption.

In the treatment of purulent effusions or empyemata we find similarly that aspiration and chemical antiseptics have been used extensively but with only mild success (17). Empyema is a most serious complication, and one should attempt to eradicate it early in order to prevent the hopeless complications, such as amyloid disease and chronic fistulae. The ultimate best treatment is thoracoplasty and is strongly urged by Hedblom (10) and Archibald (1); although we believe aspiration to be of first choice, we also believe, with Sante (19), in early thoracoplasty before the pleura can become thick and shell-like. By aspirating, the pleural pressure is kept low and the lung allowed to expand, thus facilitating thoracoplasty (3). When the empyema is the result of a mixed infection, it is much better to treat it by thoracotomy and tube-drainage previous to thoracoplasty. The former procedures are, however, absolutely contraindicated in simple tuberculous empyema.

End-results

	per cent
Artificial pneumothorax discontinued in 24 cases	33
Obliterated in 16 cases	22
Extension of disease in 8 cases	11
Amyloid disease in 6 cases	
Pleurobronchial fistulae in 5 cases	
No complications in 20 cases	
Pneumolysis in 4 cases	
Thoracoplasty in 7 cases	
Permanent drainage in 2 cases	
Economic recoveries, 11 cases	15
Improved, 18 cases	25
Total	40
Unimproved, 20 cases	28
Died, 22 cases	32
Total	60

Scanning the above figures, we can readily see how serious a problem is this effusion occurring so commonly in artificial pneumothorax. How to prevent this complication is not definitely known, but some factors stand out which deserve our attention. The existence of adhesions in 100 per cent of fluid cases is too great to be mere coincidence. They must play some part in the formation of an effusion, whether it be due to mere tension or rupture with infection of the pleura. If we could start pneumothorax in cases before such adhesions are formed perhaps we could diminish the incidence of effusions. Such a step means that we must utilize this form of collapse therapy as early as possible in the disease. Rest therapy alone has had its day and has not been satisfactory; it is

not too far-fetched to realize that the earlier and more common use of artificial pneumothorax would result in more economic recoveries, should such complications as were reviewed in this paper be reduced to a minimum. And, when such complications do appear, the pthisiologist must act, and not merely sit back and trust to Nature and rest to do the whole job. Conservatism is a great virtue and is not to be repudiated, but too long has the phthisiologist abused this *modus operandi*. In the field of tuberculous therapy more people have been allowed to die because of lack of interference than have been sacrificed by the "radical" in his experimentation with newer therapeutic agents and procedures.

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THE END-RESULTS OF THE SURGICAL TREATMENT OF PULMONARY TUBERCULOSIS[1]

PHILIP B. MATZ[2]

So much progress has been made in recent years in perfecting the various operative procedures now used in the treatment of pulmonary tuberculosis, and there has been such a marked change in the type of cases which are being selected for thoracic surgery, that it would be unfair to consider the results based upon past experience as indicative of what may be expected in the future.

In the selection of patients for surgical treatment there must be a collaboration of the internist with the surgeon; a determination must also be made of the types of lesions present, and the ratio of infection to resistance, before it is definitely decided that surgical treatment is indicated in a particular case.

In the early stages of pulmonary tuberculosis the ideal treatment is rest and the routine sanatorium regimen. If no improvement is noted following such treatment for a limited period of time, the question of whether the patient has indications for surgical treatment is next considered, and, if it is found he has, the type of operative treatment indicated in the particular case is then decided upon.

In this connection it is desired to invite attention to the experience of Fales and Beaudet in one of the Veterans Administration hospitals. These observers made a comprehensive study of the effect of rest on the healing of cavities. They found that rest appeared to be of greater importance in the healing of cavities than artificial pneumothorax, thoracoplasty, or other surgical measures. It is their opinion that in cases of cavernous pulmonary tuberculosis which are not definitely retrogressive, and without elevation of temperature, rest is the treatment of choice. If there is no tendency to heal, resort should then be had to pneumothorax or to the type of surgical procedure indicated in each case. These observers obtained complete healing of cavities in between 30 to 40 per cent of their cases following continuous bed-rest.

[1] From the Research Subdivision of the U. S. Veterans Administration. Presented at a session of the Clinical Section of the 31st annual meeting of the National Tuberculosis Association, Saranac Lake, New York, June 26, 1935.

[2] Medical and Hospital Service, Veterans Administration, Washington, D. C.

Some observers, however, hold that patients with cavernous pulmonary tuberculosis, and having good immunological response, do well under immediate surgical treatment, such as thoracoplasty. It is their opinion that valuable time is lost if bed-rest is too prolonged.

While it is possible by a set rule to determine whether or not a patient is suitable for chest surgery, it is not believed that this alone is sufficient in making a decision. There must be knowledge of the ratio of infection to resistance in the case leading to a favorable prognosis, before it is decided that chest surgery is indicated. As Dr. Harold Jessen expressed it, "Thoracic surgery must weigh and balance the extent of the whole problem, i.e., the extent of the surgical intervention against the biologic endurance of the individual patient."

Before proceeding with this report, may I say by way of introduction that of a total of 7,267 patients hospitalized for tuberculosis in Veterans Administration institutions during 1934, 571 underwent surgical treatment,—a rate of 7.9 per cent. The principal surgical regimen used was pneumothorax. Fifty-eight and three-tenths per cent of the chest surgery done consisted of induced pneumothorax; thoracoplasty, either alone or with adjunct operations, constituted 7.4 per cent of the total number of operations; phrenic-nerve operation, either alone or supplemented by other surgical procedures, constituted 32.9 per cent of the total operations; pneumolysis and pneumothorax, and oleothorax and pneumothorax, each constituted 0.7 per cent of the total chest operations.

SCOPE OF STUDY

The present study was conducted for the purpose of ascertaining the number of veterans with tuberculosis who were treated by various surgical procedures; the classification of the tuberculous disease; the extent of pulmonary involvement; the number of patients with cavities, and the size of the cavities; the effect of treatment upon the cavities; and the end-results of the surgical treatment.

These patients were under hospitalization and were treated by surgical means over a period of approximately ten years, beginning in 1924 and up to 1934. The results of their treatment were dependent upon a number of variable factors, such as the types of cases selected for surgery and the technique of the various surgical procedures used. In addition, it must be emphasized the results of the surgical treatment administered 5 to 10 years ago differ from the results obtained in the past two or three years. Inasmuch as a good deal of the chest surgery done in connection

with the present study dates back five to ten years, the earlier results are not as favorable as they would have been had the work been done more recently. An important point to bear in mind in reflecting upon the results quoted, is that, the longer surgically treated tuberculous patients are kept under observation, the greater will be the mortality rate.

TABLE 1

Classification of 320 cases of pulmonary tuberculosis treated by surgery

PULMONARY TUBERCULOSIS	NUMBER	PER CENT
Incipient A	2	0.6
Incipient B	1	0.3
Total	3	0.9
Moderately advanced A	3	0.9
Moderately advanced B	12	3.8
Moderately advanced C	1	0.3
Total	16	5.0
Far advanced A	81	25.3
Far advanced B	105	32.8
Far advanced C	115	36.0
Total	301	94.1
Grand total	320	100.0

Altogether, the study deals with 320 veterans with pulmonary tuberculosis treated by various phrenic-nerve operations, thoracoplasty, pneumolysis, or a combination of these. Fifty-six and nine-tenths per cent of the patients had had previous pneumothorax treatment, with or without success.

Two hundred and thirty-three of the 320 patients underwent some kind of phrenic-nerve operation; 80 were treated by some type of thoracoplasty; and 7 patients were treated by pneumolysis.

In the consideration of the group of 320 patients it must be emphasized that 301 of the number were in the far-advanced stages of the disease; as a matter of fact, 220 were classified as far-advanced "B" or "C"; 81 as far-advanced "A"; 16 of the 320 patients were moderately advanced; and 3 were in the incipient stage. Classification of the lesions showed that 50 per cent were predominantly productive; 23 per cent were exudative; 17 per cent were productive and exudative; and the rest of the group gave evidence of pleural lesions of one kind or another.

Further classification of the 320 tuberculous patients indicates that 22.2 per cent gave evidence of unilateral lesions; 40.9 per cent had bilateral lesions with involvement of less than one-third of the better lung; and 36.9 per cent gave evidence of bilateral lesions with involvement of more than one-third of the better lung. Two hundred and seventy-nine, or 87.2 per cent, of the 320 tuberculous patients gave evidence of cavity-formation; in 161 of the 279 cases the cavities were two inches or over in diameter.

Accordingly, the important facts to bear in mind, in evaluating the end-results of treatment of this group of cases, are that of the 320 patients 301 were classified as far advanced, and, of the latter, 242, or 80 per cent, had bilateral lesions. Furthermore, 87.2 per cent of the 320 cases gave evidence of cavities, of which number more than half were two inches or more in diameter. (Tables 2 and 3.)

TABLE 2

Classification of 320 cases of pulmonary tuberculosis treated by surgery and showing extent of involvement

PULMONARY TUBERCULOSIS	TOTAL	UNILATERAL	BILATERAL, WITH LESS THAN ONE-THIRD OF BETTER LUNG INVOLVED	BILATERAL, WITH MORE THAN ONE-THIRD OF BETTER LUNG INVOLVED
Incipient A	2	2		
Incipient B	1	1		
Total	3	3		
Moderately advanced A	3	1	2	
Moderately advanced B	12	7	5	
Moderately advanced C	1	1		
Total	16	9	7	
Far advanced A	81	21	39	21
Far advanced B	105	18	51	36
Far advanced C	115	20	34	61
Total	301	59	124	118
Grand total	320	71	131	118
Per cent	100.0	22.2	40.9	36.9

It is believed that before chest surgery was rationalized, especially in regard to the selection of cases, a good many of the tuberculous patients underwent surgical treatment as a last resort. Then, too, in many instances surgery, especially phrenic-nerve operations, was used to over-

TABLE 3

Classification of 320 cases of pulmonary tuberculosis treated by surgery and showing size of cavities

PULMONARY TUBERCULOSIS	TOTAL	CAVITIES SMALL (UP TO 1 INCH)	CAVITIES MEDIUM (1 TO 2 INCHES)	CAVITIES LARGE (2 INCHES OR OVER)	NO CAVITIES
Incipient A	2				2
Incipient B	1				1
Total	3				3
Moderately advanced A	3	3			
Moderately advanced B	12	4	1		7
Moderately advanced C	1		1		
Total	16	7	2		7
Far advanced A	81	6	23	42	10
Far advanced B	105	14	28	53	10
Far advanced C	115	11	27	66	11
Total	301	31	78	161	31
Grand total	320	38	80	161	41
Per cent	100	11.9	25.0	50.3	12.8

come some of the serious complications, such as haemorrhage, cough, excessive expectoration, etc. It is thought that with our revised present-day knowledge of the indications and contraindications, a good many of the cases in this study would not have been selected for surgical treatment.

PHRENIC-NERVE OPERATION

While there is a divergence of opinion as to the value of phrenic-nerve therapy in pulmonary tuberculosis, the preponderance of evidence seems to indicate that it is not a curative measure. It may act favorably in certain types of cases and may have a palliative effect on a good many of the disagreeable symptoms frequently encountered in tuberculous patients. It is often used as an adjunct to thoracoplasty as well as to induced pneumothorax. Of the 233 tuberculous patients in this study who were treated by phrenic-nerve therapy, 54.9 per cent had also received induced pneumothorax.

When phrenic-nerve therapy was used to overcome various disagreeable symptoms, its effect on the whole was satisfactory, particularly in lessening cough and expectoration and in the amelioration of such symptoms as fatigability and dyspnoea. In addition it decreased or stopped pulmonary haemorrhage. The effect of phrenic-nerve therapy on the

tuberculous lesions of the noncompressed lung in the case of 63 living patients appeared to be beneficial in 20.6 per cent of the cases.

In suitable cases phrenic-nerve therapy may cause either a diminution in size or a total disappearance of cavities. Forty-eight of the 63 living tuberculous patients had cavities of various sizes, 23 of which were two inches or over in diameter. These patients were benefited by phrenic-nerve therapy, as evidenced by a diminution in the size of the cavities in 31.2 per cent and a total disappearance of the cavities in 27.1 per cent; in other words, 58.3 per cent of 48 patients with cavities were favorably affected by phrenic-nerve therapy.

While the physician is interested in ascertaining whether or not phrenic-nerve operation will bring about relief of distressing symptoms and will collapse cavities, he is more interested in learning whether or not it will prolong the life of the tuberculous patient.

In reviewing the literature of phrenic-nerve therapy quite a difference of opinion is noted in the reported results. The reason for this is that physicians do not use the same procedure in selecting cases, do not use the same operative technique, and, finally, do not use a uniform terminology in reporting the results. The important point is that each case is a problem by itself, and the outcome of treatment depends upon the severity of the tuberculous disease and the immunological response of the patient. Because of these factors there is no common index which can be used in evaluating the results of treatment; therefore, such data are, strictly speaking, not comparable.

End-Results of Phrenic-Nerve Therapy

Table 4 shows the results based upon an analysis of the data in 233 cases of pulmonary tuberculosis treated by phrenic-nerve therapy. It is noted that 63, or 27 per cent, of the patients were alive at the time of the study, after periods of observation ranging from a minimum of 5 months to a maximum of 6 years and 2 months. Of the 63 cases, 35 were either much improved or improved; in 18 instances the condition of the patients was found to be stationary; and in 10 instances the patients were worse.

In connection with the 233 patients it was found that the average period between the diagnosis of tuberculosis and the data of the phrenic-nerve operation was 66.9 months, and the average survival period following the operation was 23.5 months. This includes both the living and the dead cases. The average survival period of the 63 living cases up to the date of the study was 36.1 months and the average survival period of the deceased cases was 18.8 months.

Altogether, there were 170 deaths among the tuberculous patients who underwent phrenic-nerve therapy. Pulmonary tuberculosis was the principal cause of death in 105 instances; in 60 instances pulmonary tuberculosis and other complicating diseases were the causes of death. In the remaining cases death was caused by some nontuberculous disease. These data indicate that, in spite of the phrenic-nerve operation, 165 of a total of 170 tuberculous patients died as the result of the reactivation and progression of tuberculosis.

The results following phrenic-nerve operation noted in this study indicate that, if this treatment is administered, on an average, five years after pulmonary tuberculosis is diagnosed, one cannot expect to obtain more than 50 per cent success in an effort to collapse lung cavities, particularly if these cavities are surrounded by firm, fibrous adhesions.

This study also shows that while phrenic-nerve operation does not materially save or prolong life, in a good many instances it does have a palliative effect upon disagreeable symptoms, and, as such, is of benefit to patients with advanced pulmonary tuberculosis.

Accordingly, if the beneficial effects of phrenic-nerve therapy are but temporary, the operation of choice would appear to be the crushing operation. This operation should best be done in the early stages of the disease and should not be resorted to in the later or terminal stages of pulmonary tuberculosis.

TABLE 4

Result of treatment by phrenic-nerve operation, showing the period of observation following operation (233 cases)

PERIOD OF OBSERVATION	TOTAL CASES	LIVING CASES					DIED
		Total	Much improved	Improved	Stationary	Worse	
Less than 1 month	4						4
1–6 month	34	1		1			33
7–12 months	36	1		1			35
1–2 years	61	11		7	3	1	50
2–3 years	41	17		9	7	1	24
3–4 years	25	17		8	6	3	8
4–5 years	27	13	1	6	2	4	14
5–6 years	3	2	1	1			1
6–7 years	2	1				1	1
Total	233	63	2	33	18	10	170
Per cent		27.0	0.8	14.2	7.7	4.3	73.0
Average survival period (months)	23.5	36.1	60.5	33.5	34.5	43.2	18.8

THORACOPLASTY

The purpose of thoracoplasty is to close cavities and give rest to the diseased lung in order to facilitate healing. It has been estimated that the capacity of a lung of 2400-cc. volume is diminished by 600 to 900 cc. following a thoracoplastic operation.

Until recently the technique of thoracoplasty has been in the process of modification, and the indications and contraindications of the operation have been in the course of standardization. Many patients included in this study were operated upon while in the advanced stages of the disease with lesions in the contralateral lung, with the result that the operative procedure was not followed by the best of results. In the past few years, however, the selection of the cases has been better rationalized, and the technique of the operation has been revised and perfected so that it is followed by fewer bad sequelae or complications and more favorable results.

Before the question of the use of thoracoplasty may be considered in any given case it is necessary that the physician know the type and extent of the tuberculous lesions, whether or not the tuberculous disease is progressive, and the character of the defensive power or resistance of the patient. It is essential that the resistance and recuperative power of the patient be greater than the tendency of the disease to spread, before thoracoplasty is decided upon: this is the crux of the problem.

It is most important for the tuberculosis specialist and the chest surgeon to advise thoracoplasty in the earlier stages of the disease, and not to consider it as a last-resort operation. If used in the very late stages of the disease the patient becomes a bad surgical risk and the treatment is then of little or no avail.

In the management of a case of pulmonary tuberculosis, after the patient spends a reasonable length of time at bed-rest without any apparent benefit, consideration should next be given to the possibility of administering pneumothorax or of performing thoracoplasty. There appears to be a difference of opinion as to whether it is necessary to administer pneumothorax first, and, if this regimen is found ineffective, to resort then to thoracoplasty. Some observers are of the opinion that in certain cases of pulmonary tuberculosis pneumothorax is of no avail and thoracoplasty is the operation of choice.

What was said in commenting on the end-results of phrenic-nerve operation may be repeated here, that the results at the hands of different

surgeons are not open to comparison, inasmuch as the classification of patients, the indications and contraindications used in the selection of cases, and the classification of the results of treatment, are variable and are therefore not comparable.

Statistical Data on Thoracoplasty

The present study includes 80 patients with pulmonary tuberculosis who underwent some form of thoracoplasty.

In 32 instances the surgical operation was a straight thoracoplasty; in 44 instances the technique consisted of a combination of phrenic-nerve operation and thoracoplasty; in 3 instances the operation was either a limited thoracoplasty or some other surgical measure in combination with a limited thoracoplasty; and one patient underwent a combination of phrenic-nerve operation, pneumolysis, and thoracoplasty. Fifty-eight and eight-tenths per cent of the group of 80 patients had previously been treated by pneumothorax.

Of the total number of 80 patients the oldest was 52 years of age, the youngest was 24, and the average age of the group was 34.3 years. The largest number, 63, or 78.8 per cent of the group, were between 30 and 40 years of age.

Among the 80 cases of thoracoplasty the operation was on the left side in 45 instances, and on the right side in 35 instances.

In the classification of the 80 cases of pulmonary tuberculosis according to the extent of involvement, it was found that 30 had unilateral lesions; 25 had bilateral lesions with involvement of less than one-third of the better lung; and 25 had bilateral lesions with involvement of more than one-third of the better lung.

Three of the 80 cases were classified as moderately advanced tuberculosis; and 77 were classified as far advanced tuberculosis. Sixty of the 77 patients were classified as far advanced "B" or "C".

Thirty-seven of the 80 cases had predominant productive lesions; 14 had exudative lesions; 10 had both productive and exudative lesions; the remaining number had predominant pleural lesions.

Sixty-seven, or 83.7 per cent, of the 80 cases of pulmonary tuberculosis treated by thoracoplasty gave evidence of cavitation. Ten of the number had cavities up to one inch in diameter; in 27 cases the cavities were from one to two inches in diameter; and in 30 cases the cavities were two inches or more in diameter.

While it is generally conceded that thoracoplasty is effective in closing

cavities and in arresting the tuberculous disease, nevertheless there are a number of opinions to the effect this operative procedure is not always successful.

Among 27 patients of the group who had been treated by thoracoplasty and who were alive at the time of this study, it was noted that the most favorable results following the operation were a decrease of dyspnoea and fatigability, and a reduction of the quantity of sputum. In addition, cough and expectoration were diminished and there was a slight decrease in the physical signs of the tuberculous lesions of the noncompressed lung. On the whole the palliative effects of thoracoplasty in the living group of patients were favorable.

Of the living patients who gave evidence of cavitation of the lungs, 91.3 per cent showed either a diminution in the size, or a total disappearance of the cavities following thoracoplasty.

End-Results of Thoracoplasty

The end-results following thoracoplasty reported herein are based upon data obtained in the treatment of the group of 80 cases of pulmonary tuberculosis.

It is noted that 27 of the group were alive at the time of this study after periods of observation ranging from a minimum of 4 months to a maximum of 8 years, while the average period of observation following the operation of the 27 living tuberculous patients was 33.6 months. The results of the treatment of these 27 cases show that 66.7 per cent were improved and 33.3 per cent were stationary at the time of this study.

In connection with the whole group of 80 tuberculous patients it is noted that the average interval between the date of the diagnosis of tuberculosis and the date of the thoracoplastic operation was 85.9 months; and the average survival period following the operation was 21.8 months. This includes both the living and the deceased cases. The average survival period following the operation of the 27 living cases up to the date of the study was 33.6 months; and the average survival period of the 53 deceased cases was 15.8 months.

In evaluating the results of thoracoplasty in this group, it is important to bear in mind the following facts: The operations were performed over a period of ten years beginning in 1924 and up to 1934. The surgery was done during a period when the technique was being perfected and the indications and contraindications were not standardized, nor were they applied rationally. At the present time greater care is exercised in

the selection of cases, and the chest surgeon in collaboration with the tuberculosis specialist decides whether or not the indications as well as the prognosis in a given case warrant the performance of the thoracoplasty. (Table 5.)

What was stated previously may be repeated here; namely, the longer surgically treated tuberculous patients are kept under observation, the greater will be the mortality rate.

Altogether there were 53 deaths following thoracoplasty among patients with pulmonary tuberculosis. Pulmonary tuberculosis was the

TABLE 5

Results of treatment following thoracoplasty, showing the period of observation following operation (80 cases)

PERIOD OF OBSERVATION	TOTAL CASES	LIVING CASES			DEAD
		Total	Improved	Stationary	
Less than 1 month	7				7
1–6 months	15	4	2	2	11
7–12 months	16	3	2	1	13
1–2 years	12	5	4	1	7
2–3 years	8	1		1	7
3–4 years	11	6	4	2	5
4–5 years	7	5	4	1	2
5–6 years	1	1		1	
6–7 years	1				1
7–8 years	1	1	1		
8–9 years	1	1	1		
Total.....	80	27	18	9	53
Average survival period (months).....	21.8	33.6	35.9	29.2	15.8

principal cause of death in 22 instances; in 31 instances pulmonary tuberculosis and other complicating diseases were the causes of death. Operative shock was the direct or contributory cause of death in 6 instances; cardiac disease was either the direct or contributory cause of death in 9 instances; pulmonary haemorrhage was either the primary or contributory cause in 3 instances; and pneumonia was a contributory cause of death in 6 instances.

Seven of the 53 cases died within one month of the thoracoplasty; 5 died between one and two months following the operation; 13 died from seven to twelve months after the operation; 7 died from one to two years

after the operation; and 7 of the number died from two to three years after the operation. The time of death of the remaining cases may be seen by referring to table 5. It is therefore plain that in spite of the thoracoplasty there was an extension of the tuberculous disease which resulted in the death of these patients.

INTRAPLEURAL PNEUMOLYSIS

The consensus of opinion seems to be that in the treatment of pulmonary tuberculosis, pneumothorax therapy should be given a trial for at least 3 to 6 months in an effort to collapse the lung, because frequently adhesions may stretch sufficiently so that cavities will close. If a complete collapse is not possible by ordinary pneumothorax, the question of intrapleural pneumolysis to break up adhesions should next be considered.

Matson claims that 45 per cent of his cases of partial pneumothorax are suitable for pneumolysis. On the other hand O'Brien states that the need for pneumolysis seldom arises. Kremer is of the opinion that pneumolysis is done in about 8 per cent of cases of artificial pneumothorax. Pomplum has performed intrapleural pneumolysis in 14 per cent of his cases.

Statistical Data on Intrapleural Pneumolysis

The present study includes 7 patients who were treated by means of intrapleural pneumolysis. Five of the patients were classified as chronic pulmonary tuberculosis, far advanced "A"; 2 were classified as chronic pulmonary tuberculosis, far advanced "B". The youngest was 30 years of age at the time of the operation; the oldest was 44 years of age; and the average age for the group was 36 years. Of the 7 cases, pneumolysis was performed on the left side in 6 instances, and on the right side in 1 instance. Five of the patients had bilateral lesions with involvement of less than one-third of the better lung; 2 of the patients had unilateral lesions.

Six of the patients had fibrotic tuberculous lesions as well as extensive pleural adhesions; one gave evidence of pleural adhesions only. In addition to these lesions, 4 of the patients had medium-sized cavities (one to two inches in diameter); 3 of the patients had large-sized cavities (two inches or more in diameter).

A study of the interval of time between the diagnosis of pulmonary tuberculosis and the date of the performance of intrapleural pneumolysis shows that the shortest period was six months; the longest period was six years; and the average period was 35.4 months.

All of the 7 patients had been subjected to pneumothorax therapy which did not result in complete collapse, thus necessitating the use of intrapleural pneumolysis. The average interval of time between pneumothorax and pneumolysis was 7.9 months. In 6 instances pneumothorax was continued following intrapleural pneumolysis.

A study of the effects of intrapleural pneumolysis in six of the group of seven patients showed that it had no effect on the physical signs of the noncompressed lung in 4 instances; in one instance there was a decrease of the physical signs; and in one instance there was an increase of the physical signs of the noncompressed lung.

The effect of intrapleural pneumolysis in collapsing cavities showed that 4 of the 6 patients gave evidence of a disappearance of the cavities;

TABLE 6

Results of treatment following intrapleural pneumolysis showing the period of observation following operation (7 cases)

PERIOD OF OBSERVATION FOLLOWING OPERATION	TOTAL CASES	LIVING CASES				DEAD
		Total	Much improved	Improved	Stationary	
1–6 months	1					1
1–2 years						
2–3 years	2	1			1	1
3–4 years	3	1	1			2
4–5 years	1	1		1		
Total.....	7	3	1	1	1	4

Longest period, 4 years; shortest period, 4½ months; average period, 31.6 months.

in one instance there was a decrease in the size of the cavities; and in one instance the pneumolysis had no effect whatever on the cavities.

Of the 7 patients treated by intrapleural pneumolysis 4 showed the presence of complications; 2 gave evidence of suppurative pleurisy; and 2 of hydropneumothorax.

Results of Treatment

Of the 7 tuberculous patients treated by intrapleural pneumolysis, 3 are alive and 4 are dead. Of the 3 living patients, one is much improved, one is improved, and the condition of the third case is stationary.

A study of the survival period of patients following intrapleural pneumolysis indicates that the longest survival period was 4 years; the shortest was four and one-half months; and the average survival period following the operation was 31.6 months.

No conclusions may be drawn from the figures cited because of the small size of the group. However, of the 6 patients on whom follow-up data were available, 4 gave evidence of the disappearance of cavities; in 1 case the cavities were decreased in size; and in 1 case no effect was noted in the size of the cavities following the operation. Inasmuch as the purpose of intrapleural pneumolysis is the conversion of an unsatisfactory collapse to a satisfactory one, and inasmuch as the operation was effective in attaining such a result in 4 of 6 cases, it may be said that intrapleural pneumolysis proved to be beneficial in the treatment of this group of cases. (Table 6.)

SUMMARY AND CONCLUSIONS

1. This study deals with 320 veterans treated for pulmonary tuberculosis by phrenic-nerve operations, thoracoplasty, pneumolysis, or a combination of these operative procedures. One hundred and eighty-two, or 56.9 per cent, of the patients had had previous pneumothorax treatment, with or without success.

2. Two hundred and thirty-three of the 320 patients underwent phrenic-nerve operation; 80 were treated by some type of thoracoplasty; and 7 received intrapleural pneumolysis.

3. In evaluating the end-results of the surgical treatment of the 320 tuberculous patients it is important to bear in mind the fact that 301 of the total number were classified as far advanced, and, of the latter, 242, or 80 per cent, had bilateral lesions; 87.2 per cent of the 320 cases had cavities. Classification of the lesions showed that 50 per cent were productive; 23 per cent exudative; and 17 per cent exudative and productive lesions; in the remaining number the lesions were pleural.

4. When phrenic-nerve therapy was used to overcome some of the disagreeable symptoms its effect was satisfactory, particularly in lessening cough and expectoration and in ameliorating such symptoms as fatigability and dyspnoea. It also decreased or stopped pulmonary haemorrhage.

5. Phrenic-nerve therapy resulted in either a diminution in size or in a disappearance of cavities in 58.3 per cent of a total of 48 living patients who gave evidence of cavity-formation.

6. Sixty-three, or 27 per cent, of a group of 233 patients treated by phrenic-nerve therapy were alive at the time of the study, after periods of observation ranging from a minimum of five months to a maximum of six years and two months. Thirty-five of the 63 cases were either much

improved or improved; in 18 instances the condition of the patients was found to be stationary; and in 10 instances the patients were worse.

7. The data of this study indicate that, in spite of the phrenic-nerve operation, 165 of a total of 170 tuberculous patients died as the result of the progression of tuberculosis. It would appear that phrenic-nerve therapy does not save nor does it materially prolong life. In many instances phrenic-nerve operation was applied for its palliative effect; in such cases it had a beneficial effect in that it ameliorated the disagreeable symptoms.

8. Eighty of the group of 320 patients underwent thoracoplasty. In the classification of the 80 cases of pulmonary tuberculosis according to the extent of involvement it was found that 30 had unilateral lesions; 25 had bilateral lesions with involvement of less than one-third of the better lung; and 25 had bilateral lesions with involvement of more than one-third of the better lung. Three of the 80 cases were classified as moderately advanced and 77 were classified as far-advanced tuberculosis. Sixty of the tuberculous patients were classified as far advanced "B" or "C". Sixty-seven, or 83.7 per cent, of the 80 cases of pulmonary tuberculosis treated by thoracoplasty gave evidence of cavitation. Thirty of these cavities were two inches or more in diameter. Thirty-seven of the 80 cases had predominantly productive lesions; 14 had exudative lesions; and 10 had both productive and exudative lesions; the remaining number had pleural lesions predominant.

9. Twenty-seven of the 80 patients treated by thoracoplasty were alive at the time of this study. It was noted that the most favorable results following the operation were a decrease of dyspnoea and fatigability, and a reduction of the quantity of sputum. In addition, cough and expectoration were diminished, and there was a slight decrease in the physical signs of the tuberculous lesions of the non-compressed lung. Of the living patients who gave evidence of cavitation of the lungs, 91.3 per cent showed either a diminution in the size or a total disappearance of the cavities following thoracoplasty.

10. The twenty-seven patients with pulmonary tuberculosis who had been treated by thoracoplasty and who were alive at the time of this study were observed for periods ranging from a minimum of 4 months to a maximum of 8 years. The average period of observation of these patients was 33.6 months. The results of the treatment of these cases shows that 66.7 per cent were improved; and 33.3 per cent were stationary at the time of the study.

11. Fifty-three tuberculous patients died following thoracoplasty. It was found that in spite of the thoracoplasty there was an extension of the tuberculous disease which resulted in the death of the patients.

12. Seven patients of the total group of 320 were treated by means of intrapleural pneumolysis. Five of these patients were classified as chronic pulmonary tuberculosis, far advanced "A"; and 2 as far advanced "B". The 7 patients had been subjected to pneumothorax therapy which did not result in complete collapse, thus necessitating the use of intrapleural pneumolysis.

13. The effect of intrapleural pneumolysis in collapsing cavities showed that 4 of the 6 patients on whom progress information was available gave evidence of disappearance of cavities; and in one instance there was a decrease in the size of the cavities; in but one of the cases was the pneumolysis unsuccessful. Three of the patients who underwent pneumolysis were alive and 4 were dead at the time of the study.

14. It has been shown that the life expectancy of patients with active advanced pulmonary tuberculosis is short. It has been found by several investigators that 90 per cent of these patients are dead within 5 years of the appearance of positive sputum, with treatment either inside or outside of sanatoria. Accordingly, if surgical collapse will reduce the death rate from 90 to approximately 70 per cent in a group of cases such as the ones cited, then there is justification for its use. If, as is now being shown by many chest surgeons and tuberculosis specialists, by a careful selection of cases in the earlier stages and using an improved technique, the results will be more satisfactory, and the mortality rate can be still further reduced, then there should be no hesitancy in using the various surgical collapse measures in the treatment of advanced pulmonary tuberculosis.

OBSERVATIONS ON TUBERCULOSIS WORK IN GERMANY

H. E. KLEINSCHMIDT

Leaders of German and American tuberculosis work discussing their scientific problems around one table would be a most congenial group. Their attitudes toward the problem of controlling tuberculosis and their epidemiological experiences would be much alike, and these they would discuss in a technical language common to all, save for a few differences in terminology. This should not be surprising, for always in the past we have been peculiarly responsive to Germany's thought in matters pertaining to tuberculosis,—the discoveries of Koch, the empiricism of Dettweiler, the deductions of Ranke, and many other advances found ready acceptance in this country.

To-day Germany's public-health scheme is being rebuilt to fit the pattern of her new political philosophy, and this involves to a considerable extent the programme to combat tuberculosis. The new German political concept is somewhat like this: To fulfill her destiny, it is argued, Germany the Nation, potentially great but slowly being crushed to death, must become Germany the Race. The old Germanic blood must be freed from the infusions of foreign bloods; Nordic culture must be restored and purified. Furthermore, the population age-curve is not in Germany as it should be in a healthy nation, pyramidal in form, but inscribes an outline like an urn which means that a relatively large proportion of middle-aged people are being supported by narrower bands of young people and children. Should the present trend continue, Germany would die out in a few centuries. Hence the emphasis on race hygiene reflecting itself in public health in the so-called eugenics laws, which provide for the sterilization of the biologically unfit, and also in the effort to increase the birth-rate of acceptable racial stock by encouraging marriage and the rearing of children by means of liberal bounties and appeals to patriotism.

But Germany's problem is complicated by immediate economic factors as well as long-distance considerations of race. Cut off from the world she must learn to sustain herself. All her resources must be carefully husbanded, new ones must be created. She feels that she must build

herself into a compact, complete unit, independent of the rest of the world. That means economy of her greatest wealth, man-power. Health and happiness, not of a favored few, but of all her people, is the goal.

Public health is committed to these two principles, namely, the betterment of the racial stock (as interpreted by Party leaders) and the equable distribution of social wealth. The achievement of the goal requires the control of medicine by the State, rigid organization of all resources, and regimentation of all groups.

ADMINISTRATION OF TUBERCULOSIS WORK

The administrative set-up for health protection is still somewhat obscure, if not tangled. There seem to be overlapping of duties, differing interpretations of regulations, and confusion as to the relationship of one department toward another. This is to be expected. Two flags fly to-day[1] in Germany, and each has its counterpart in the governmental machinery. The Reich programme is still operative but the programme of the Party is rapidly being grafted into it. Welfare enterprises, which were and still are supported by voluntary contributions, are now enjoying (or enduring) semiofficial status. For example, there has been organized an Extension Bureau under the aegis of the Ministry of the Interior which has taken over various nonofficial health organizations including the German Central Committee for Combating Tuberculosis, now known as the *Reich Tuberkulose Auschuss* (Imperial Tuberculosis Extension). Officers of the discontinued Committee have retired, and the administrative head of the new organization, Dr. Denker, is responsible directly to the Minister of the Interior. The purpose of the new tuberculosis extension is to coördinate and superintend all tuberculosis work and to make it part of the public-health programme. As rapidly as possible the Imperial Tuberculosis Extension will be subdivided into 13 district bureaus. Membership is voluntary, though limited to persons sympathetic with the new government, and dues are paid as before.

The general policy of the new Tuberculosis Extension is reflected in a statement of its first annual report of 1933–1934, as follows:

Not medical care (Fürsorge) for the weak, casualties in life's battle, but husbandry of the heritable wealth shall, from now on, be the motto. The old

[1] This paper was accepted for publication before the announcement of the Swastika as to be henceforth the single national emblem of the new Reich.

and often misleading concept of medical care has too often conveyed a false picture of the fight against tuberculosis, as if our main responsibility were to succor the individual patient. Protection of the well is hereafter to occupy first place and this purpose is served also by cure of the sick. Medical care, sanatorium treatment of sufficient duration and isolation of the seriously ill are measures just as necessary as the prompt removal of children from contact, and education in health protection.

Another agency dealing with tuberculosis is the National-Socialistic Public Welfare Department of the party commonly known as the *N.S.V.* It seeks to ensure medical care for the individual patient, to eliminate the sources of infection, to find cases not likely to be discovered by other agencies, and to provide relief and care for those cases unprotected by social insurance carriers or welfare bureaus. It is attempting to coördinate the efforts of the many unrelated provisions for the tuberculous, such as health centres, welfare agencies, student health services, panel doctors and the like. If a patient needs sanatorium treatment and has no means of securing it, if travel is necessary, if clothing is required, if the family of the patient needs looking after, the *N.S.V.* steps into the breach. The claim is made that no one in Germany to-day who has tuberculosis need suffer neglect. Before the "Revolution" thousands of persons with tuberculosis were living in garden houses, tiny summer shelters on the outskirts of every city and wholly unfit for continuous occupancy; but this deplorable situation no longer exists.

It is difficult for the visitor to Germany to understand fully how these two organizations stand in relation to each other. The emphasis of the *Tuberkulose Auschuss* seems to be on the promotion of tuberculosis-control as a subdivision of public health, while the *N.S.V.* seems particularly concerned with the social and medical aspects of the tuberculosis problem.

In addition to these executive organizations there is the German Tuberculosis Association, comparable to our American Sanatorium Association and consisting largely of sanatorium physicians and technicians. There is also the Association of German Tuberculosis Physicians whose membership includes clinicians specializing in tuberculosis.

An example of well coördinated machinery for the control of tuberculosis in a local community may be seen in the seaport Stettin on the Baltic, a city of about 250,000 population. The entire scheme is under the sole direction of Professor Bräuning. The clinic is located in an old but well-furnished building in the congested part of the city. The nurs-

ing sisters, all of whom are well trained, serve in the clinic and carry on the follow-up work in the homes. The sanatorium, situated in a fragrant pine forest about one-half hour by train from the city, is simple but adequate. Certain patients, generally married people without children, are cared for in selected residences in the city and maintained by public funds and they are, of course, carefully supervised. This type of domiciliary care has proved to be practical and takes a considerable burden off the sanatorium. Routine fluoroscopic examinations of groups such as Soldiers, Arbeit's Front Units (comparable to our CCC and WPA groups), college and school students, are part of the programme. Case-finding of the usual kind among tuberculosis contacts is so thoroughly organized that one wonders how any citizen with tuberculosis could escape discovery. Professor Bräuning also supervises the education of the general public and the teaching of tuberculosis prevention in the common schools. In collaboration with an educator he has written a textbook for teachers which includes suggestions on teaching methods. As early as 1912 he made a study of what children actually know about tuberculosis and what they can be taught. The basic idea that underlies the whole scheme and which is constantly impressed upon the people is that tuberculosis is a communicable disease, transmission of which takes place most commonly in the household. Health camps for children and mothers were at one time a part of the scheme, but these have been discontinued because it was found they did not justify the expense. Painstaking attention to detail is everywhere evident. In the sanatorium, for example, every eating utensil and toilet article is numbered and each patient uses only those assigned to him. This is, of course, troublesome and unnecessary, since all eating utensils are sterilized, but, says Professor Bräuning, why not let the patient practice what we preach? The sanatorium is a training school, and an essential part of the training is to learn to use only one's own toilet and eating utensils. Who can escape such German logic!

In Westphalia one sees a fine example of tuberculosis control in a difficult rural area centering around Brilon-Wald Sanatorium under the able direction of Dr. Koester. The county it serves is irregular in shape, mountainous, and sparsely populated. Forestry and farming are the chief occupations. Communication is difficult, for highways are poor and railroads are circuitous. The sanatorium is beautifully located, satisfyingly complete in equipment and attractively decorated. The rooms are commodious, and the porches on the South command a magnificent view of typical German woods and valleys. X-ray and surgical

apparatus are of the latest design. A fluoroscope, equipped with a foot-controlled turn-table on which the patient stands, enables the operator to move the patient easily in any desired position. A special feature is the obstetrical department including a nursery for babies born at the institution. This section of the sanatorium occupies a wing entirely separated from the rest of the building. Patients are carefully taught before the baby is born how to care for the child and especially how to safeguard him against infection. Mothers nurse and bathe their own babies, wearing while doing so a cellophane face mask which covers the nose and mouth. (Incidentally, the tiny droplets of sputum which the mother sees plainly on the mask when she removes it make a powerful impression on her.) Thus far not a single baby has been infected before leaving the hospital. The sanatorium is the focal point from which case-finding work in the county radiates. Contacts and arrangements are made in advance by a nursing sister who is thoroughly acquainted with the neighborhood. Motor transport carries a portable, specially designed X-ray apparatus. The sanatorium superintendent or one of his assistants conducts the clinics. Local doctors bring their patients, and join the examining physician in determining whether the patient should be sent to the hospital or continue under the observation of the local doctor. Parents are urged to bring in their infants for a tuberculin test. Older children and young people are examined in groups with the fluoroscope. Since 1929 there has been a consistent drop in mortality, morbidity and infection rates; in 1934 only 11.5 per cent of tested school-children reacted to tuberculin.

MASS EXAMINATIONS

Throughout Germany, group examination of apparently healthy persons is recognized as an established case-finding measure, not, to be sure, as a substitute for the examination of tuberculosis contacts but as a valuable supplement. Dr. F. Redeker is largely responsible for this development. His interest in tuberculosis was born of experience in the Ruhr Valley in the terrible years after the war when he was commissioned to examine thousands of children and found tuberculosis to be the most common threat of childhood. He found at that time that while only 20 per cent of infants reacted to tuberculin, the source of infection of 90 per cent of these could be definitely traced, whereas 80 per cent of urban children aged 8 to 13 years reacted to the tuberculin test but the infection source could be found in only 20 per cent of them.

For the tuberculin test the Von Pirquet technique seems to be generally

preferred, and next in favor is the Moro method, modified as to formula and technique to suit the preferences of individual workers. It is freely admitted that the intracutaneous test is more precise, but because many people object to the hypodermic needle and not the scratch or ointment test, a degree of precision is sacrificed for the advantage of reaching a greater number of people.

For X-ray work paper film is in common use. German workers were driven to the use of paper film by economic necessity after the war and now have in the course of years developed a very satisfactory product which costs about 20¢ for a single flat picture of the chest. It is said by some to equal the celluloid film.

Fluoroscopy is in wide use. For group examinations it is used much as we employ the tuberculin test, namely, as a screen to pick out those apparently healthy persons whose lungs require further study. The writer is not a roentgenologist but it seemed to him that the apparatus used is more efficient than that commonly seen in the United States. In spite of the physical strain on the examiner German phthisiologists willingly and as a matter of course use this instrument more freely than we seem to in this country. To fluoroscope 40 patients at a sitting is not considered an over-arduous task. Speed and economy are the reasons given for the rather widespread use of fluoroscopy in group examinations. A general idea of the findings may be gained from results of routine examinations of a series of 4,000 workmen gathered in camps for the unemployed. The findings were classified as follows:

Open, communicable tuberculosis	3.2 per 1,000
Active tuberculosis (not immediately communicable)	4.5 per 1,000
Inactive tuberculosis	6.6 per 1,000
Calcified nodules	62.0 per 1,000
Pleural or diaphragm changes	13.7 per 1,000

OPINION ON MOOT SUBJECTS

Concerning questions currently discussed in America it appears that the consensus of German opinion (based on conversations with some 15 phthisiologists) corresponds closely with what one may call majority opinion in this country. For example, BCG is viewed with considerable skepticism. Two or three persons of high reputation volunteered the opinion that the disaster at Lübeck was undoubtedly due to transmutation of the bacillus in culture, though that could not be proved in court.

Gold therapy is discussed with tongue in cheek, the feeling being that

sanocrysin and other gold derivatives are very well for cases of good prognosis but not a worthy substitute for the tried and tested methods in common use, and far too expensive.

Lung surgery is everywhere practiced. Nerve operations to immobilize the diaphragm are rapidly losing in favor while pneumothorax is being extensively used, especially in earlier cases, and fewer contraindications are being respected than was formerly the case. Upper-rib thorocoplasty has its enthusiastic promoters, some insisting that the risk is minimal and the result more certain than pneumothorax.

What we in this country commonly designate as health education is probably not so well done in Germany. With some local exceptions, as noted in Stettin, for example, the art of shaping public opinion, creating a demand for modern facilities and stirring the public conscience, is not highly developed. Such printed matter as is here and there issued is usually dull and not attractively printed. A motion picture made under the direction of Dr. Klare, showing the activities of Scheidegg, a sanatorium for children, stirred some enthusiasm, but somehow neither the movie nor other popular educational and publicity devices play a real part in the campaign. At the annual business meeting of the Tuberkulose Ausschuss attended by about 300 workers from all parts of Germany there was shown, as an example of an American movie for interesting children and their parents, the film called "The Story of My Life by Tee Bee," which is a fantastic but telling presentation of the basic facts of tuberculosis. The minutes of this meeting stated, "The trick drawings are, according to our taste, somewhat grotesque but certainly very picturesque and effective." Several persons in the audience after the meeting remarked that the movie was "not serious enough,—our people would only laugh and forget." On the other hand, samples of American popular booklets and posters exhibited at the meeting, brought approbation and expression of the wish that such material might also be available for Germany. However, education of the patient in the sanatorium is given the same meticulous attention as medical treatment. Through individual instruction, rigid discipline and good example a patient learns the rules for recovery and health maintenance.

In one department of popular health education German workers far outstrip their American colleagues and that is visual education by means of exhibits. The German Hygiene Museum at Dresden is of course world famous—a splendid example of a people's university, specializing in hygiene and biology. In the spring of 1935 there was held in Berlin a

health exposition called "Das Wunder Des Lebens." A description of that masterly enterprise appears elsewhere.[2] During its six weeks operation it was visited by approximately three-fourths of a million people. This exhibit, as well as the several smaller ones which are moved from place to place, reveal excellent teaching technique. They are designed primarily to teach the fundamental biological facts underlying health and disease by means of apparatus worked by the visitor, models and demonstrations. Doubtless the German people respond more readily to "reason why" education than we do in this country; nevertheless the "laboratory" or exhibit method as developed in Germany might advantageously be cultivated more seriously in this country.

The ascent of the Party is probably responsible, indirectly at least, for a very present danger. While cultists, quacks and "nature healers" have always thrived in Germany they have merely been tolerated by law and by the profession, as in this country, as medical outcasts. Now, through Party channels, they are asserting themselves and demanding official recognition. Recently some extremists in the Party urged that German medical science be purged of all Jewish influence including past discoveries of men like Wassermann, Ehrlich and Von Behring. Even Koch, among many others, was denounced as being tarred with the Jewish stick, because Jews had been employed as assistants in his laboratory. Cooler heads in the government at once met this challenge with counter-propaganda and issued a strong statement on the anniversary of Robert Koch's birth, eulogizing him and pointing out the greatness of his work. It seems evident that there will be a struggle to keep charlatans and fanatics out of sanatoria, hospitals and the official health direction.

Strange as it seems Friedmann, notorious exploiter of turtle serum, still roams at large and carries on his nefarious propaganda. No way has yet been found to silence him. Fresh from the press is an exhaustive treatise of 284 closely written pages exposing "Friedrich Franz Friedmann and his Tuberculosis Remedy." It includes a graphic description of that gentleman's whirlwind sweep in the United States, his publicity conquest of several other countries, his present activities in Germany. His "science" is thoroughly picked to shreds as only German investigators can do it—yet he seems to thrive and wax fat. Tuberculosis workers complain that it is impossible to exercise control over many

[2] Journal of Public Health, October, 1935.

active carriers and spreaders of the disease because they claim they are under Friedmann's treatment and therefore refuse the help or advice of officials. The author of the book, Dr. Alfred Knapp, hopes that "the unassailable evidence he has produced may cause the authorities to devise the necessary means of holding Friedmann responsible for the great harm he has done at home and abroad, to the reputation, the wealth and the blood of the German people."

Naturally the future of public health and tuberculosis work in Germany is bound up as it is in America, with the fate of the country as a whole. Intense nationalism, the driving force of the Third Reich, if it does not lead to war, may develop a strong self-sustaining nation, but doubtless at the sacrifice of a metropolitan exchange of goods and ideas with the rest of the world. If she succeeds some day in achieving an isolated independence, it will probably mean also impoverishment, economically and culturally for all her people. Happily, medicine, particularly that part of it which has to do with disease prevention, is a common denominator of the world least likely to be influenced by political philosophy. It is encouraging too to note that in the present national turmoil the leaders in the medical profession are keeping their heads and quietly resist the tendency to be stampeded into any extreme position. We shall continue to hope therefore that Germany may not lose her place as a leader and inspirer in the worldwide fight against tuberculosis.

THE COST OF TUBERCULOSIS TO INDUSTRY, TO THE INDIVIDUAL AND TO THE COMMUNITY[1]

WILLIAM ALFRED SAWYER[2] AND EDWARD KARL RICHARD[3]

APPROACH TO THE STUDY

The study about to be presented consists of a detailed analysis of all the costs involved in 100 cases of tuberculosis, occurring among the employees in the Rochester, New York, plants of the Eastman Kodak Company since 1922. These cases were chosen without regard to the stage of the disease.

Such an investigation as this involves not only the costs of diagnosis, treatment, and after-care, but the loss of wages resulting from protracted disability. In a study of this character the many varied and complex factors are immediately evident. (Appendix A.)

This study was begun on September 15, 1932, and after more than two and one-half years of usage and experience, no essential changes or additions to the general "set-up" have been required.

MATERIAL, PERSONNEL, AND FACILITIES AVAILABLE FOR STUDY

A distinct aid in conducting a study of costs of tuberculosis in groups of individuals is an accurate case-record covering a period of years during which direct observations have been chronologically entered. Certain industrial groups present ideal material, and such material has been selected for this study.

In addition to having available complete case-records kept over a period of years, the existence of adequate personnel, and facilities, further aided the investigation. Engrossed as they are with routine work, community medical-service agencies, which might have at their disposal tuberculosis case records for financial study, are not usually prepared to shoulder the further burdens involved in a cost-analysis. They may be, and usually are, handicapped by lack of funds and insufficient personnel. These obstacles are largely surmountable in certain industrial groups.

[1] Presented at a Session of the Administrative Section of the 31st annual meeting of the National Tuberculosis Association, Saranac Lake, New York, June 26, 1936.

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In strictly medical agencies the usual lack of continuous availability of reasonably large groups of persons for study makes prolonged direct observation difficult, if not impossible. The scattering and interruption of supervision received by any group of tuberculous persons is a familiar phenomenon to phthisiologists. This has been demonstrated by experience in following sanatorium ex-patients in our community (1). It was found that, whereas four-fifths of them were diagnosed in the offices of private practitioners, only one-fifth of them returned to such offices after receiving treatment at a distant institution. They either returned to no supervision at all, or to public or semipublic medical supervision, scattered through many different agencies. A study of costs on such a group of tuberculous individuals in the community would necessitate the creation of a separate correlating agency of investigation.

Finally, on the cheerful coöperation of all interested individuals and agencies must depend the successful completion of any fact—gathering effort. This has been secured and is gratefully acknowledged. We wish particularly to express our gratitude to Miss Elizabeth Copeland, R.N. and Mrs. Bertha Thayer, R.N. for the painstaking care with which they assembled the mass of detailed information upon which this paper is based; and to Miss Ruth MacIntosh, of the medical staff of this industry, we extend our sincere thanks for her untiring and helpful assistance in the many details encountered.

TABLE 1

100 cases of tuberculosis in industry
Accuracy of report

Per cent accuracy	100%	95%	90%	85%	80%	75%	70%
Number of cases	49	4	29	7	5	5	1

THE STUDY

The cases used were taken at random from the files of an industry where case-finding and follow-up were complete and thorough. The medical care and treatment in each case were adequate when the coöperation of patient and family was secured, which was our common experience.

The accuracy of each case analyzed, was rated in terms of per cent (table 1). The Sick Benefit records provided complete and accurate data because all sickness is followed regularly. In some instances it was difficult to get information from individuals and institutions, and the

figures had to be estimated. A tabulation of the accuracy of the reports revealed the following: 49 of the cases were rated as 100 per cent accurate, 40 from 85 to 95 per cent, and 11 from 70 to 80 per cent.

Whether such a small number of carefully studied case-records will result in findings of sufficient value upon which to base conclusions, remains to be seen. In any event, the value of the investigative method here used may reasonably be determined, and this may constitute to some extent a minor purpose of this contribution.

UNITS OF COST

In a study of the costs involved in the care of groups of such tuberculous persons, it is essential for the purposes of accuracy, to reduce to statistically usable "units of cost" all such factors as (1) the types of service required; (2) the frequency with which such services are required; and (3) the allocation of financial obligations incurred in the fulfilling of those requirements. Since each case-record in this tuberculous group presents such a varied array of these factors, it is evident that, without the establishment of specific "units of cost," a study of this kind would be quite futile. (*Appendix A* describes the technical steps followed in arriving at the "units of cost" used in preparing the master fact-gathering sheet, table 2, on which the findings in this study are based.) A brief glance at table 2 reveals that the "units of cost" for services and losses of varying types are listed in the vertical column to the extreme left. It will be noted that the agencies rendering such services extend horizontally across the left-hand portion of the master sheet, broadly divided into "Private" and "Public" agencies, and further subdivided as you see. The costs of such services by allocation, as distributed between the agencies, are listed horizontally across the right-hand portion of the sheet. Further information appearing on the master sheet was found to be essential for the completion of the study.

The calculations used in filling in the "Life Expectancy" bracket appearing on the master sheet were based on the life-expectancy figures as used by the Metropolitan Life Insurance Company in 1932; namely, 56 years for men, and 58 years for women. In view of the fact that the workers studied were not exposed to any unusual occupational hazard, it was not felt that their expected mortality was any different than that of the general population. The social, industrial, and economic status of the workers was referred to in a paper read before this Association in 1931.

From such a broad angle of approach as has just been described, it is

Present Status:
Date of Study

TABLE 2

Accuracy of Reports:

TUBERCULOSIS COSTS BY INDUSTRY

Name..... Plant.....Department.....
Address..... Born.....Sex.....
Date of Diagnosis..... Date of Employment.....

State of Disease on Diagnosis							Result of Treatment						
Service Unit	Service rendered by (write in name)						Costs by Allocation						TOTALS
	Private			Public			Private			Public			
	PRIVATE — M.D.	INDUSTRY	VOL. AGENCY	LOCAL	STATE	FEDERAL	FAMILY	INDUSTRY	VOL. AGENCY	LOCAL	STATE	FEDERAL	
Clinical Exams. and Reports													
X-Ray													
Visits M.D.													
Nursing Visits													
Social Service													
Hospital Days Lost													
Working Days Lost													
Sick Benefit													
Estimated Time Lost													
Contact Cases													
Life Expectancy			TOTAL COSTS BY ALLOCATION										
Stopped Work													
Returned													
Average Weekly Wage													

apparent that, for the purposes of the study, there was no such thing as "free care" for these tuberculous individuals. Someone invariably has had to "foot the bill."

In view of the falling mortality figures for tuberculosis, over the past thirty-five years, it is not uncommon to find public-health officials lukewarm toward an active programme against tuberculosis. Either they consider the problem solved, or sufficiently controlled to need no further intensive effort. To be sure, the death-rate has fallen from first to seventh position for all age-groups in the past generation in the U. S. Registration Area. However, an analysis of the statistics reveals the fact that for women in the age-groups, 20–24 and 25–29, the death-rates are still 110 and 108 per hundred thousand, respectively, which, as Whitney (2) points out, is as high as it was fourteen years ago for the entire population. For males, the death-rate runs 74 per hundred thousand, and it is the second highest cause of death among men, exceeded only by heart disease.

Tuberculosis still takes its heavy toll in those early years when young people are establishing themselves in their life work. Prolonged disability at such an age is not only economically devastating at the time, but is often a handicap throughout life.

Because of the waste involved in the protracted disability, it seems worthwhile to determine the economic values involved. In industry, where everything is measured in terms of money, the cost of tuberculosis is naturally of interest. Therefore, these one hundred cases were analyzed.

Our chief object in this study is to call attention to the economic aspect of tuberculosis because it is our belief that by so doing it will be possible to focus renewed attention upon the importance and necessity of dealing with the problem more actively. So far as we know, no effort has been made to evaluate economically the results of tuberculosis in an industrial group. Perhaps this study will arouse new interest in endeavoring to approach the so-called irreducible minimum.

FINDINGS

This study concerns 49 women and 51 men. The average age at the time of diagnosis in the women was 25.9 years, and in the men it was 36.7 years, giving an average for both sexes of 31.3 years (table 9—appendix B).

The average length of disability for the women was 542 working days; for the men, 648 (table 3). The average for the two sexes was 595, or

TABLE 3

100 cases of tuberculosis in industry
Average length of disability—working days

	APPARENTLY ARRESTED	SATISFACTORY	TOTALLY ARRESTED	QUIESCENT	UNIMPROVED	TOTALLY DISABLED	DIED	UNKNOWN	AVERAGE DAYS BY DIAGNOSIS
Women—49 cases									
Minimal A	388	237	628	1,100	1,231	—	401	166	428
Advanced	417	702	—	—	—	—	1,171	—	802
Far advanced	1,434	—	—	—	520	—	326	—	652
Tuberculous peritonitis	—	—	487	—	—	—	558	—	523
Average days by result of treatment	456	337	592	1,100	994	—	829	166	542
Number in each group	17	14	4	1	3	—	9	1	49
Men—51 cases									
Minimal A	395	558	384	—	—	2,992	523	—	578
Advanced	303	488	—	3,160	1,349	—	571	—	821
Far advanced	211	—	—	—	580	—	570	—	499
Tuberculous peritonitis	—	262	—	—	—	—	—	—	262
Average days by result of treatment	346	510	384	3,160	1,221	2,992	566	—	648
Number in each group	17	12	2	1	6	1	12	—	51

TABLE 4

100 cases of tuberculosis in industry
Result of treatment

	PULMONARY TUBERCULOSIS			TUBERCULOUS PERITONITIS	TOTAL	
	Minimal A	Advanced and moderately advanced	Far advanced			
Apparently arrested	22	9	3	—	34	66 per cent
Satisfactory	18	7	—	1	26	
Thoroughly arrested	5	—	—	1	6	
Quiescent	1	1	—	—	2	
Unimproved	2	5	2	—	9	31 per cent
Totally disabled	1	—	—	—	1	
Died	2	9	9	1	21	
Unknown	1	—	—	—	1	
Total	52	31	14	3	100	

two years and fifteen weeks, a very considerable time to be spent in enforced idleness. Of the entire group, 66 per cent has been recorded as arrested, 31 per cent as unimproved or dead (table 4). This leaves

2 per cent rated as quiescent, on the basis of their ability to work, and 1 per cent unknown (unable to estimate cost beyond severance of connection with the company because patient moved away, and case was lost to further follow-up). Among the women 71.5 per cent were classi-

TABLE 5

100 cases of tuberculosis in industry
Allocation of cost by service units

	MINIMAL A	ADVANCED AND MODERATELY ADVANCED	FAR ADVANCED	TUBERCULOUS PERITONITIS	TOTAL	PER CENT	
Clinical {reports, examinations}	$961	$543	$207	$92	$1,803	0.4	
X-ray	1,062	696	192	107	2,057	0.5	
Visits—M.D	1,978	1,691	474	485	4,628	1.1	
Nursing visits	2,132	1,035	457	143	3,767	0.9	
Social service	701	656	300	273	1,930	0.5	
Hospital days	28,074	26,608	8,515	2,549	65,746	16.2	
Working days lost	51,233	39,671	10,419	4,193	105,516	26.0	
Sick benefit	36,620	34,946	10,063	1,153	82,782	20.4	79.8
Estimated time lost	43,765	66,607	25,306	—	135,678	33.4	
Contact cases	825	507	920	3	2,255	0.6	
Total	$167,351	$172,960	$56,853	$8,998	$406,162	100.0	
Number of cases	52	31	14	3	100		
Average cost per case by stage of disease	$3,218	$5,578	$4,061	$2,999	$4,062		

TABLE 6

100 cases of tuberculosis in industry
Minimum and maximum costs

	MINIMAL A	MODERATELY ADVANCED AND ADVANCED	FAR ADVANCED
Minimum	$311	$612	$114
Maximum	$24,508	$15,889	$16,773
Average	$3,218	$5,578	$4,061

fied as arrested, and 24.5 per cent as unimproved or dead. Among the men 60.7 per cent were arrested, and 37.3 per cent were unimproved or dead (tables 10 and 11—appendix B).

The entire cost of diagnosis, treatment, and lost time from work, including the examination of contacts, was $406,162. This gives an

average of $4,062 per case (table 5). (By sex in appendix B.) It is interesting to note the great extremes in costs of these cases (table 6). The far-advanced case costing $114 died nine days after the diagnosis was made. The fact that one case of tuberculosis, even though minimal, can cost as much as $24,508 is worthy of serious thought.

The costs have been analyzed in two ways: first, as to the services necessary in diagnosis, treatment, follow-up, and lost time; second, as to the allocation of costs between private and public agencies involved in the handling of these cases.

A glance at the table of service costs (table 5) will show that the major cost is wages lost and Sick Benefit paid during disability (Appendix D—Range of Weekly Wage). This amounted to 79.8 per cent of all service expense. The length of disability was calculated on the basis of ability to return to work. Working days lost includes the time from the first day absent to the date when the individual either returned to work or severed connection with the company. Estimated time lost indicates the length of disability subsequent to severance of connection with the company to life expectancy. An adjustment was made in the calculations of wages lost and amount of money paid in Sick Benefit. In each case Sick Benefit was subtracted from wages lost.

Clinical examinations, visits of doctors and nurses, social service, days in the hospital, and examination of contacts amounted to 20.2 per cent of the cost (table 5) (Appendix D—Range of Service Costs).

The complexity of the problem of service to tuberculous individuals, and the difficulty of following such cases is demonstrated by the fact that 93 different doctors and agencies were involved in the care of these 100 cases, and the total number of initial visits or contacts with these different doctors and agencies was 355 (table 7) (Appendix C). It is obvious that the total number of initial and subsequent contacts with these doctors and agencies would produce an imposing total. We are unable to give you this figure accurately at this time.

The entire cost of this study was borne by the Company and is not included in our calculations.

In the allocation of costs, according to agencies rendering services (table 8) (by sex in Appendix B), 79 per cent was spent by private sources, 63.2 per cent of this being charged to the family. Public agencies spent only 21 per cent. Thus, it is evident that the largest burden falls on the individual or family. In this particular study, sick individuals were forced to spend a total of $256,690. This is certainly a

TABLE 7

100 cases of tuberculosis in industry
Analysis of doctor and agency coöperation

	NUMBER	NUMBER OF INITIAL CONTACTS
Private		
Family		
Doctors	58	87
Hospitals	9	26
Industry		
Nursing service	1	2
Sick benefit	1	100
Voluntary agency	7	16
Public		
Local	8	80
State	3	33
Federal	6	11
Total	93	355

TABLE 8

100 cases of tuberculosis in industry
Allocation of cost by private and public agencies

	MINIMAL A	ADVANCED AND MODERATELY ADVANCED	FAR ADVANCED	TUBERCULOUS PERITONITIS	TOTAL	PER CENT
Private						
Family	$101,323	$114,276	$35,351	$5,740	$256,690	63.2
Industry	29,636	23,440	7,212	1,330	61,618	15.2
Voluntary agency	567	1,285	46	407	2,305	0.6
Total private	131,526	139,001	42,609	7,477	320,613	79.0
Public						
Local	$10,039	$13,625	$5,832	$1,521	$31,017	7.6
State	12,169	5,112	1,043	—	18,324	4.5
Federal	13,617	15,222	7,369	—	36,208	8.9
Total public	35,825	33,959	14,244	1,521	85,549	21.0
Total: public and private	$167,351	$172,960	$56,853	$8,998	$406,162	100.0

strong argument for some sort of sickness insurance, because few individuals are able to stand the expense of such a long-term illness. It is our contention that this cost is higher than for most diseases, at least than for any communicable disease.

There are few communicable diseases that disable individuals for two years and fifteen weeks, which is the average for the group in this study. Of course, it is perfectly evident that the earlier cases are discovered, the less they will usually cost, as was shown in the recent study by Seder (3).

If tuberculosis is to be practically wiped out, as a few of the communicable diseases have been, will it not be necessary to think more in terms of its cost to individuals and to society? Now that people are economically-minded and there is more scrutiny of medical costs, is the time not ripe for including in our educational propaganda information relative to the expenses and waste involved in the detection and care of tuberculosis? Loss of wages now means more than ever. Prolonged disability for young manhood and young womanhood is of such vital concern to everyone that it deserves the fullest consideration.

CONCLUSION

Whereas the National Tuberculosis Association has proved through an analysis of case histories by Seder that an adequate expenditure in money in the early diagnosis and treatment of tuberculosis is a paying investment, we have endeavored to establish the fact, by an economic analysis of 100 cases of tuberculosis in an industrial group:

1: That tuberculosis is a costly disease, more so than any other communicable disease, the average cost in our series being $4,062. (A single case had cost over $24,000, even though minimal when diagnosed.)

2: That tuberculosis takes its toll in the early years of earning a livelihood when an individual can least afford to be idle and pay the cost of illness, and

3: That tuberculosis should be more carefully considered and studied in its economic aspects in planning our future educational programmes.

Perhaps this contribution will lead to similar studies of other communicable and disabling diseases. Only by such comparison and analysis will we be able to solve some of the problems involved in the prevention and possible eradication of these diseases. Such studies should be helpful in deciding some of the important questions concerning the cost of medical care, helping us to reach conclusions regarding a better distribution and arrangement of such costs.

SUMMARY

1. The investigative method used for studying the cost of tuberculosis as it occurred among one hundred employees of an industry (The East-

man Kodak Company of Rochester, N. Y.), in which there is no particular respiratory hazard, was found to be practical and comprehensive. Appendix A describes the method used as demonstrated by the master fact-gathering sheet (table 2). The costs considered in this particular study include the examination of contacts.

2. Essentials for the successful completion of this study or any similar analysis of this type were found to be:

A: Accurate case records covering a period of years during which *direct observation* had been chronologically noted.

B: Adequate, intelligent personnel and facilities.

C: Complete coöperation of all individuals and agencies concerned. In other words, community good will was of vital importance.

3. The important findings resulting from this study, which was begun in 1932, are as follows:

A: The average time loss due to tuberculosis was 595 working days, or 2 years and 15 weeks.

B: The total cost involved in caring for these 100 cases was $406,162.00.

C: The average cost per case was $4,062.00.

D: The average cost by stage of disease was Stage 1, $3,218.00; Stage 2, $5,578.00; and Stage 3, $4,061.00.

E: The maximum cost per case by stage of disease was Stage 1, $24,508.00; Stage 2, $15,889.00; and Stage 3, $16,773.00.

F: The minimum cost per case by stage of disease was Stage 1, $311.00; Stage 2, $612.00; Stage 3, $114.00.

G: Private agencies bore 79.8 per cent of the costs incurred, of which the family bore 63.2 per cent ($256,690.00). Public agencies (tax-supported) bore 20.2 per cent.

H: Private agencies rendered 79 per cent of required services; public agencies 21 per cent.

I: The average age of the group was 31.3 years for the 49 women and 51 men concerned.

Appendices with tables are included in the published paper for the benefit of those desiring more detailed information concerning the findings in this study.

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Appendix A

As has been noted: to successfully conduct a cost-analysis such as this study represents with respect to the economic factors concerned in the care of tuberculosis patients, the varied items concerned had to be standardized into statistically usable "units of cost."

In order to determine these varied "units of cost," it was necessary to analyze what the determinants of these "units" might be. A survey of the facts in their broader aspects revealed that the determinants of these "units of cost" fell into three major groups as follows: Group 1 embraced Medical Determinants; group 2, the Auxiliary Determinants; and group 3 the Financial Determinants. These were considered briefly in outline, and, second, were considered fully in detail. Not until this was done was it possible to determine the actual "units of cost" usable for this particular study of the cost of tuberculosis in an industrial group.

OUTLINE OF DETERMINANTS OF UNITS OF COST

Medical Determinants

The nature of clinical tuberculosis determines the types of service, frequency of services, and length of service required in each instance. For purposes of analysis these were subdivided according to the Phases of Tuberculosis as follows:

1. The Phases of Tuberculosis as Medical Determinants of the "Units of Cost"

A: The phase of diagnosis (stage of disease on diagnosis)

B: The phase of treatment (result of treatment)

1: (a) Apparently arrested
(b) "Satisfactory"—(family doctor's report)
(c) Thoroughly arrested
(d) Quiescent

2: Unimproved

3: Died

4: Unknown

C: The phase of after-care (working days or time lost)

D: The phase of case-finding (contact examinations)

Auxiliary Determinants

The following factors were auxiliary to the main course of medical supervision and financial management encountered in each case-record studied. For purposes of consideration they are categorically listed as follows:

2. Auxiliary Determinants of "Units of Cost"

A: Clinical examinations and reports
B: X-ray service
C: Visits by physician
D: Nursing visits
E: Social service visits
F: Hospital days
G: Working days lost
H: Sick benefit
I: Estimated time lost
J: Contact cases examined
K: Necessary miscellaneous information:
  1: Life expectancy
  2: Stopped work: date
  3: Returned to work: date
  4: Average weekly wage

Financial Determinants

The protracted course of tuberculous disease, even in its earliest and most favorable stages, makes shared or divided allocation of the financial obligations incurred the rule in most cases, rather than the exception. For purposes of discussion these have been listed as follows:

3. Financial Determinants of "Units of Cost"

Private Financial Determinants

1: Voluntarily supported sources:
  A: The family (individual) assumed total, partial, no obligation
  B: The employing industry assumed total, partial, no obligation
  C: The community volunteer agencies assumed total, partial, no obligation

Public Financial Determinants

2: Tax-supported sources:
  D: The local tax-supported agencies assumed total, partial, no obligation
  E: The state tax-supported agencies assumed total, partial, no obligation
  F: The federal agencies assumed total, partial, no obligation

COMMENT ON THE OUTLINE OF DETERMINANTS OF "UNITS OF COST"

Contemplation of the three broad groups of Determinants of "Units of Cost" show that groups 1 and 2 (Medical and Auxiliary Determinants) were cross-influenced throughout by group 3 (Financial Determinants). This being true, it was obvious that, for an analysis of the factors operating in the case of any given individual, it would simplify the tabulation of collected facts to have the Medical and Auxiliary Determinants occupy the position of ordinates and to have the Financial Determinants occupy the position of abscissae on the cross-hatch of the statistical table as used (see table 2). In table 2 it will be noted that the full cost of *any given type of service* rendered to the tuberculous individual in the group under consideration will be found in the last column to the right. This sum will represent the addition of the shared allocations of financial obligations incurred by the agencies appearing horizontally across the top of the table. Similarly, the financial contribution of each agency to the total care of the particular case under consideration will be obtained by addition of the vertical column in each instance. Finally, the cost of any group services, such as medical, nursing, institutional, etc., may be obtained by adding both vertical and horizontal columns containing items included within the specified brackets (see extreme right and left margins of table 2).

As is characteristic of all vital (human) statistics, a certain percentage of error or inaccuracy was to be expected. In many instances the actual "Units of Cost" used represent estimates, often colored by personal opinion, rather than actual costs. Such partial inaccuracy, however, would be common to any study of this kind, which by its nature must deal with intangible factors as well as with tangible factors.

For convenience and accuracy duplicate sets of the master fact-gathering sheets (table 2) were made, one to remain in the individual's case-record, the other to be placed in the statistical files of the study.

Appendix B

FURTHER TABLES ON STUDY OF COSTS

TABLE 9

100 cases of tuberculosis in industry
Average age at time of diagnosis

	MINIMAL A	ADVANCED AND MODERATELY ADVANCED	FAR ADVANCED	TUBERCULOUS PERITONITIS	AVERAGE BY RESULT OF TREATMENT	NUMBER OF CASES
			Women—49 cases			
	years					
Apparently arrested	27.5	23.5	23.0	—	26.3	17
Satisfactory	23.8	23.7	—	—	23.8	14
Thoroughly arrested	27.0	—	—	21.0	25.5	4
Quiescent	20.0	—	—	—	20.0	1
Unimproved	23.5	—	20.0	—	22.3	3
Totally disabled	—	—	—	—	—	—
Died	34.0	33.6	25.5	21.0	30.4	9
Unknown	27.0	—	—	—	27.0	1
Average by diagnosis	25.8	27.7	23.5	21.0	25.9	49
			Men—51 cases			
Apparently arrested	36.1	37.4	29.5	—	35.7	17
Satisfactory	43.2	33.5	—	32.0	39.0	12
Thoroughly arrested	33.0	—	—	—	33.0	2
Quiescent	—	31.0	—	—	31.0	1
Unimproved	—	40.2	49.0	—	41.7	6
Totally disabled	31.0	—	—	—	31.0	1
Died	26.0	35.8	35.6	—	34.8	12
Unknown	—	—	—	—	—	—
Average by diagnosis	37.4	36.6	35.7	32.0	36.7	51

TABLE 10

Tuberculosis in industry

Men—51 cases

Results of treatment

	PULMONARY TUBERCULOSIS			TUBERCULOUS PERITONITIS	TOTAL
	Minimal A	Advanced and moderately advanced	Far advanced		
Apparently arrested	10	5	2	—	17
Satisfactory	7	4	—	1	12
Thoroughly arrested	2	—	—	—	2
Quiescent	—	1	—	—	1
Unimproved	—	5	1	—	6
Totally disabled	1	—	—	—	1
Died	1	4	7	—	12
Total	21	19	10	1	51

TABLE 11

Tuberculosis in industry
Women—49 cases
Results of treatment

	PULMONARY TUBERCULOSIS			TUBERCULOUS PERITONITIS	TOTAL
	Minimal A	Advanced and moderately advanced	Far advanced		
Apparently arrested	12	4	1	—	17
Satisfactory	11	3	—	—	14
Thoroughly arrested	3	—	—	1	4
Quiescent	1	—	—	—	1
Unimproved	2	—	1	—	3
Died	1	5	2	1	9
Unknown	1	—	—	—	1
Total	31	12	4	2	49

TABLE 12

Tuberculosis in industry
Men—51 cases
Allocation of cost by service units

	MINIMAL A	ADVANCED AND MODERATELY ADVANCED	FAR ADVANCED	TUBERCULOUS PERITONITIS	TOTAL	PER CENT
Clinical (reports, examinations)	$437	$389	$175	$8	$1,009	0.4
X-ray	448	438	141	74	1,101	0.4
Visits—M.D	1,353	969	207	175	2,704	1.0
Nursing visits	1,208	601	337	43	2,189	0.8
Social service	541	549	40	90	1,220	0.4
Hospital days	10,170	17,269	7,321	274	35,034	12.8
Working days lost	22,830	26,286	6,213	781	56,110	20.6
Sick benefit	27,007	30,227	9,234	555	67,023	24.5
Estimated time lost	35,118	48,642	21,081	—	104,841	38.4
Contact cases	583	414	920	—	1,917	0.7
Total	$99,695	$125,784	$45,669	$2,000	$273,148	100.0

TABLE 13

Tuberculosis in industry
Women—49 cases
Allocation of cost by service units

	MINIMAL A	ADVANCED AND MODERATELY ADVANCED	FAR ADVANCED	TUBERCULOUS PERITONITIS	TOTAL	PER CENT
Clinical {reports, examinations}	$524	$154	$32	$84	$794	0.6
X-ray	613	258	50	33	954	0.7
Visits—M.D.	625	721	267	310	1,923	1.4
Nursing visits	924	434	120	100	1,578	1.2
Social service	160	107	260	183	710	0.5
Hospital days	17,904	9,339	1,194	2,275	30,712	23.1
Working days lost	28,403	13,385	4,206	3,412	49,406	37.1
Sick benefit	9,613	4,719	830	598	15,760	11.9
Estimated time lost	8,648	17,965	4,225	—	30,838	23.2
Contact cases	242	94	—	3	339	0.3
Total	$67,656	$47,176	$11,184	$6,998	$133,014	100.0

TABLE 14

Tuberculosis in industry
Men—51 cases
Allocation of cost by agencies

	MINIMAL A	ADVANCED AND MODERATELY ADVANCED	FAR ADVANCED	TUBERCULOUS PERITONITIS	TOTAL	PER CENT
Private						
Family	$60,713	$81,994	$26,653	$1,137	$170,497	62.4
Industry	18,192	17,892	6,182	611	42,877	15.7
Voluntary agency	114	1,049	1	—	1,164	0.4
Total	79,019	100,935	32,836	1,748	214,538	78.5
Public						
Local	$1,561	$6,535	$4,634	$252	$12,982	4.8
State	5,498	3,092	830	—	9,420	3.4
Federal	13,617	15,222	7,369	—	36,208	13.3
Total	20,676	24,849	12,833	252	58,610	21.5
Total: public and private	$99,695	$125,784	$45,669	$2,000	$273,148	100

TABLE 15

Tuberculosis in industry
Women—49 cases
Allocation of cost by agencies

	MINIMAL A	ADVANCED AND MODERATELY ADVANCED	FAR ADVANCED	TUBERCULOUS PERITONITIS	TOTAL	PER CENT
Private						
Family	$40,610	$32,282	$8,698	$4,603	$86,193	64.7
Industry	11,444	5,548	1,030	719	18,741	14.1
Voluntary agency	455	236	45	407	1,143	0.9
Total	52,509	38,066	9,773	5,729	106,077	79.7
Public						
Local	$8,477	$7,090	$1,198	$1,269	$18,034	13.6
State	6,670	2,020	213	—	8,903	6.7
Federal	—	—	—	—	—	—
Total	15,147	9,110	1,411	1,269	26,937	20.3
Total: private and public	$67,656	$47,176	$11,184	$6,998	$133,014	100

Appendix C

HOSPITALS, SANATORIA AND AGENCIES INVOLVED

Hospitals and Sanatoria

Monroe County Tuberculosis Sanatorium
New York State Hospital for Incipient Pulmonary Tuberculosis (Raybrook)
St. Mary's Hospital
Municipal Hospital
Strong Memorial Hospital
Rochester General Hospital
Trudeau Sanatorium
Sullivan's Camp
State Hospital for Insane
Violet Hill Sanatorium, North Carolina
Eagle Bay Hospital
Rainbow Sanatorium
Bennington, Vermont Sanatorium

Veterans Hospitals

Aspinwall
Sunmount
Tupper Lake
No. 98—Castle Point

Agencies

G. A. R.
Industrial workshop
Monroe County Tuberculosis Dispensary
Rochester Tuberculosis Association
State Rehabilitation Bureau
Metropolitan Nursing Service
Public Health Nursing Association
Department of Charities
Family Welfare
City Welfare
Community Chest
Government Compensation
Metropolitan Life Insurance Company
Connecticut Mutual Life Insurance Company
Town of Gates
Town Relief—Irondequoit
Costa Rican Government

Appendix D

RANGE OF SERVICE COSTS

"Unit of Cost"	*Range*
Clinical examinations	$1.00–$3.00
Clinical reports	$1.00–$3.00
X-rays—single film	$1.00–$5.00
stereo	$2.00–$8.00
Visits—M.D. private	$1.50–$15.00*
M.D. voluntary agency	$1.22
Nursing visits	$1.00–$2.00
Social Service—in hospital	$.62
Hospital days	$2.75–$4.85
Weekly wage	$15.00–$100.00†

* 64 per cent Private M.D. visits ranged from $2.00 to $3.00. 2 single items of $25.00 and $30.00 appeared in Private M.D. visits.

† Majority in lower wage-levels.

AN INEXPENSIVE SYNTHETIC MEDIUM FOR GROWING MYCOBACTERIUM TUBERCULOSIS

SAM WONG[1] AND JOHN WEINZIRL[1]

INTRODUCTION

For some years past the Alice McDermott Foundation has employed Synthetic Medium Tuberculin as one of its principal antigens in experiments relating to desensitization of tuberculous animals. For the production of this tuberculin we have used exclusively Long and Seibert's synthetic medium containing asparagine for growing tubercle bacilli. The following factors have made it desirable to find a cheaper synthetic medium for growing the organisms: (1) the high cost of asparagine, approximately $25 per pound; (2) the desirability of employing a synthetic medium in the preparation of the many tuberculin antigens; and (3) the increased use of protein antigens in desensitizing experiments. If a less expensive substitute can be found it would be highly desirable, especially if it also affords a more rapid growth.

With the exception of Henley and LeDuc's synthetic medium which contains ammonium malate, we have not found any synthetic medium prepared in recent years in which the relatively expensive asparagine is not used. Henley and LeDuc's medium (1) was therefore tried. After repeated attempts we were unable to grow *Mycobacterium tuberculosis*, H37, with any degree of consistency and luxuriance. Three difficulties were encountered in using this medium: (1) the glucose is caramelized during the autoclaving of the medium, thus imparting to it a dark color and adding an uncertain factor in the changed glucose; (2) after autoclaving at 12-pounds' pressure for 20 minutes as specified, the reaction of the medium is usually sufficiently acid to inhibit the growth of the organisms; (3) a precipitate is always formed, a qualitative analysis of which showed that it consists of ferric-phosphate and magnesium-ammonium-phosphate. The precipitation of the phosphates removes much of the buffering power as well as some of the necessary food materials in the medium. The purposes of the following experiments were (1) to prepare a medium which will be as successful for growing tubercle bacilli

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as Long and Seibert's medium; (2) to obtain a less expensive medium; and (3) to increase the growth-rate of the tubercle bacilli.

REVIEW OF LITERATURE

The chief objection to Long and Seibert's medium is the relatively high cost of one of its constituents, namely, asparagine. It is evident that a substitute for the chemical is highly desirable. Wherry (2) reported that ammonium chloride can be used as a source of nitrogen when monopotassium-phosphate and sodium chloride are also present. Kendall, Walker and Day (3) employed diammonium-hydrogen-phosphate as the source of nitrogen and phosphorus. Merrill (4) used ammonium sulphate as a source of nitrogen in his study of the carbon metabolism of the tubercle bacillus. These sources of nitrogen, however, are of experimental significance only, since all of them are decidedly inferior to asparagine. Most synthetic media, employed for the production of tuberculin, contain asparagine as a source of nitrogen. A review of these media is unnecessary, since a discussion of Long and Seibert's and of Henley's articles relating to their media is representative of the entire group.

Long and Seibert (5) were able to obtain 100 to 150 gm. of moist tubercle bacilli, type H37, per litre of medium in four to six weeks. Henley (6) reported that the presence of dextrose increased the growth-rate of the tubercle bacilli on his asparagine medium. He found that one per cent of glucose gave the lower limit for maximum growth, and that 4 per cent or higher inhibited the growth. Other sugars such as maltose, xylose, arabinose, and galactose had little or no effect on the growth-rate of the bacilli. Henley and LeDuc (1) found that the ammonium salt of malic acid showed an efficiency approaching that of asparagine. By replacing asparagine with ammonium malate they obtained a medium which yielded 15 to 19 gm. of bacteria, dry weight, per litre of culture-fluid in about 6 weeks, in contrast to 20 gm. obtained in Henley's asparagine medium for the same period of time. The ammonium salts of carbonic, maleic, aspartic, citric, fumaric, lactic, tartaric, and succinic acids are all decidedly inferior to asparagine. To quote the authors: "In no case was the growth more than approximately half as great as that obtained from the regular asparagine medium."

METHODS EMPLOYED

Organism used: Rapidly growing cultures of *Mycobacterium tuberculosis*, strain H37, were used throughout the study.

Growth requirements: In order to study the minimum requirement of each constituent in the medium, the chemical in question was omitted in the preparation of the culture-fluid. It was then introduced aseptically in varying amounts. The minimum amount which gave the maximum yield was retained for the final medium.

Preparation of the media: Long and Seibert's medium was prepared according to directions given by the authors. The composition and procedure for preparing the modified medium are as follows:

Modified Long and Seibert's Medium

Malic acid	3.00 gm.
Ammonium hydroxide (10 per cent solution)	12.00 cc.
Ammonium citrate	5.00 gm.
Monopotassium acid phosphate	6.00 gm.
Sodium carbonate (anhydrous)	2.00 gm.
Sodium chloride	2.00 gm.
Magnesium sulphate	1.00 gm.
Ferric ammonium citrate	0.05 gm.
Glycerol	50.00 cc.
Distilled water, to make	1000.00 cc.
Glucose solution to be added after sterilization	10.00 gm.

Ingredients may be dissolved by means of heat if necessary. The initial pH should be 6.8 to 7.0, the final pH about 7.0. The medium was autoclaved at 20 pounds' pressure for 15 minutes. A 20 per cent c. p. dextrose solution is sterilized in test-tubes to facilitate introduction into the flasks. The amount placed in each tube is such that when added to the medium the final concentration is approximately one per cent. If it becomes necessary to readjust the pH after autoclaving the medium must be titrated to pH 7.4 and autoclaved again at 20 pounds' pressure for 15 minutes. Apparently reautoclaving does not alter the usefulness of the medium.

RESULTS OBTAINED

Maximum growth of *Mycobacterium tuberculosis*, H37, on the modified medium was obtained in about four weeks and on Long and Seibert's in about seven weeks. If one per cent of glucose is added to the latter medium after it has been autoclaved, maximum growth is obtained in about 24 days, or approximately half of the time. However, if the glucose is added to the medium and then autoclaved maximum growth is not obtained until after five or six weeks. Evidently the changed glucose has an inhibitory effect on the organism. The optimum concentration of glucose for the modified medium ranges from 0.5 to 6 per cent.

In order to show the growth-rates of H37 on the two different media the organisms were grown for three weeks. The amount of protein present in the culture fluid was measured by Seibert's method, employing trichloracetic acid as the precipitating agent. The weight of organisms was determined by filtering the culture through asbestos in a Gooch crucible and drying to constant weight in an oven at 100°C. For safety in handling, all the flasks were sterilized by streaming steam for 20 minutes at 100°C. The results given in table 1 represent the averaged values obtained in three different experiments:

TABLE 1

Growth of mycobacterium tuberculosis, type H37, at the end of three weeks

MEDIA	NUMBER OF CC. TAKEN	PRECIPITATE IN HOPKIN'S TUBE IN CC.	MILLIGRAMS OF PROTEIN PER LITRE OF MEDIUM	GRAMS OF BACTERIA PER 200 CC. OF MEDIUM
Long and Seibert's (modified)	10	0.010	67.4	2.07
Long and Seibert's	10	0.004	27.0	1.40

The results show clearly the rapid rate of growth on the glucose medium, as contrasted with Long and Seibert's medium. We realize that if the organisms had been allowed to continue their growth to the maximum, the results would have been different; nevertheless, this fact does not invalidate the advantage of more rapid growth obtained on the non-asparagine medium over that on the asparagine medium. A photograph of the cultures taken at the end of the third week of growth is presented (figure 1) to show the relative amounts of growth on the different media. Representative results from each group of cultures were taken for the picture.

The flasks shown in figure 1 were originally inoculated from a two-weeks-old culture of H37 with approximately the same amount of organisms and incubated at 37°C.

The costs per litre of medium as calculated on the basis of the price quoted per pound of c. p. chemicals, as taken from the 1934 catalogue of the Coleman and Bell Company, are as follows: Long and Seibert's medium, 36 cents, and modified Long and Seibert's, 10 cents. The greater cost of Long and Seibert's medium is due to the asparagine.

All the available Mycobacterium species and varieties in our laboratory have been successfully grown on the ammonium malate medium. These include the following: *Mycobacterium tuberculosis*, H178, a culture of low virulence for guinea pigs, from Hygienic Laboratory, Washington, D. C.;

Mycobacterium tuberculosis, H1721, from the Michigan Department of Health; *Mycobacterium tuberculosis*, H520, from British Columbia University; *Mycobacterium tuberculosis*, A407, from Oregon State College; *Mycobacterium bovis*, T1658, from the University of California; and the nonpathogens from the Phipps Institute: (1) *Mycobacterium butter*, Type V; (2) *Mycobacterium butter;* (3) *Mycobacterium Korn*, Type V; (4) *Mycobacterium mist;* (5) *Mycobacterium phlei;* (6) *Mycobacterium*

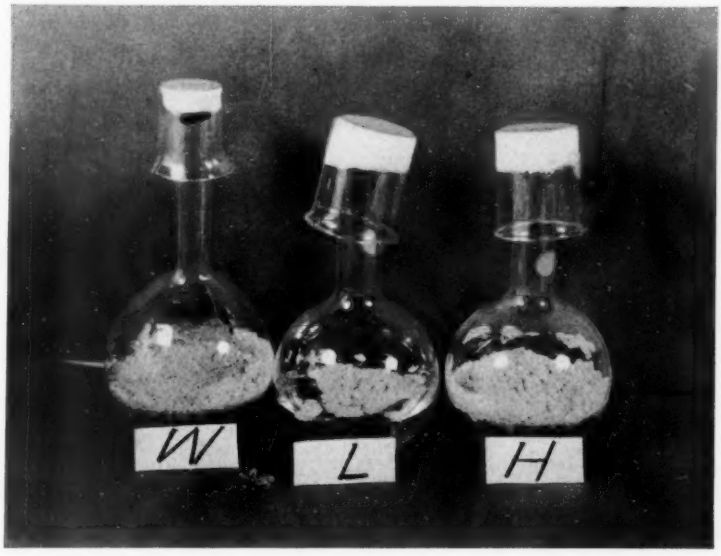

FIG. 1. Growths of organisms at the end of the third week in (W) modified Long and Seibert's medium; (L) Long and Seibert's medium; (H) modified Henley and LeDuc's medium.[2]

smegmatis. Apparently any *Mycobacterium* which will grow on Long and Seibert's asparagine medium will also grow on the ammonium-malate medium.

DISCUSSION

Maximum growth of *Mycobacterium tuberculosis*, H37, in the ammonium-malate medium without the addition of glucose was obtained in about six weeks. The growth-rate, however, was not as uniform as

[2] The modified Henley and LeDuc's medium is used interchangeably with the modified Long and Seibert's medium. It is comparatively more difficult to prepare and is not reported here.

when glucose was present. It may be noted that when glucose was present, the modified Long and Seibert's medium showed a striking difference in appearance from the other medium; the culture-fluid at the time when the maximum growth was reached presented a reddish color, resembling that of the asparagine medium which had been standing for a long time. The pigment was absorbed by the collodion membrane employed in ultrafiltering the proteins for the production of synthetic medium tuberculin.

The exact nature in which glucose is utilized to effect this increase in the growth-rate of H37 is not known. Merrill (7), Gamble and Herrick (8), and we (unpublished results) have shown that glucose is metabolized by H37. Further, we have found that glucose in proper concentration is a satisfactory substitute for glycerol for the growth of *Mycobacterium tuberculosis*, H37. Analyses are in progress at present to study the tuberculoprotein obtained from a glycerol-free medium prepared by us. This and other related phases of metabolism will be reported in another paper.

The glucose synthetic medium has been in use in the Alice McDermott Foundation for the past two years for growing all types of Mycobacterium. We have found that the tuberculoprotein produced from the modified medium is apparently identical with that from the asparagine medium of Long and Seibert's. A comparison of the skin tests made on tuberculous guinea pigs with 0.005 mgm. of the two tuberculoproteins showed no difference in reaction.

SUMMARY AND CONCLUSIONS

1. An inexpensive synthetic medium is presented which is suitable for the production of tuberculin in large quantities. Its production is approximately one-third the cost of that for Long and Seibert's asparagine medium.

2. Maximum growth of *Mycobacterium tuberculosis*, H37, in the ammonium-malate medium, with one per cent glucose added, may be obtained in about four weeks, or about the same weight of organisms may be obtained in approximately half of the time usually required.

3. The same amount of tuberculin can be obtained with the glucose-malate medium as with the asparagine medium, but in approximately one-half the time.

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THE RÔLE OF CHRONIC VITAMIN-C DEFICIENCY IN THE PATHOGENESIS OF TUBERCULOSIS IN THE GUINEA PIG[1, 2]

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INTRODUCTION

Vitamin-C deficiency results in a well-known clinical syndrome of scurvy and the experimental form of this disease may be produced at will in the laboratory. However, scurvy represents an end-stage, the clinical manifestation of a gross deficiency. Frequently, the human diet is partially inadequate in its vitamin content (Yavorchy, Almaden, and King, 1934), and it is of interest to ascertain whether or not a chronic deficiency in this vitamin may not influence the course of a bacterial infection. The effect of complete and partial vitamin-C deficiency on resistance to tuberculosis has been studied by numerous investigators. The basic components of the diet have not been the same as the deficient one, the difference in vitamin-C content being only one of the variables. The experiments have been conflicting. Further study seems warranted before the rôle of vitamin C in experimental tuberculosis is conclusively demonstrated.

Prausnitz and Schilf (1924) found in a group of 3 scorbutic and 4 nonscorbutic guinea pigs infected with tuberculosis that the tuberculin reaction was weakened or became negative in the scorbutic animals. Leichtentritt (1924) gave large amounts of orange juice to tuberculous guinea pigs on normal diets and found the survival period to be twice as long as that of tuberculous animals on a normal diet alone. The latter animals developed a miliary type of infection as contrasted with an encapsulated process in the orange-juice group.

Bieling (1924) placed a group of animals, which had had chronic tuberculosis for two years, on a scurvy-producing diet and found that they died earlier than did the noninfected scorbutic guinea pigs. Recently infected scorbutic animals did not die sooner than the controls. When a partially depleted vitamin-C diet was given to chronic tuberculous ani-

[1] From the Pediatric Research Laboratory, the Jewish Hospital of Brooklyn, New York.
[2] This work was made possible by a grant from Mead Johnson and Company.

mals, death was delayed; but these animals also died earlier than did noninfected, chronic scorbutic animals. It appeared that the development of scurvy was accelerated by tuberculosis. Bieling also found (1925) scorbutic tuberculous animals to be more susceptible to subcutaneous injection of tuberculin than similarly infected animals receiving vitamin C. Mouriquand, Dochaix and Dosdat (1925) showed the tuberculous process to advance more rapidly in guinea pigs on a diet producing chronic scurvy than on a control diet. Chronic vitamin-C-depleted animals died in half the time of those receiving a normal diet, following the inoculation of tubercle bacilli, according to the experiments of Heymann (1926). Basu (1934) found the mortality of guinea pigs inoculated with tubercle bacilli to be higher in those on a ration deficient in vitamin C than in the group receiving a vitamin-complete diet.

McCarrison in 1919–1920 noted punched-out ulcers in the duodenum of scorbutic guinea pigs. Microscopically, the mucosa showed atrophic and necrotic changes and loss of villus structure. Mackie and Chitre (1928–29) showed superficial sloughing of the mucous membrane and, at times, a more severe ulceration in the large intestine of monkeys. These animals were partially depleted of the scorbutic vitamin.

Medlar and Sasano (1924) studied the serial sections of the grossly negative guts of guinea pigs inoculated subcutaneously with tubercle bacilli. These animals lived 65–145 days. Tuberculous lesions were found in the large majority of the group. In 1922, Redman followed the development of tuberculosis in animals who were fed infected sputum once or twice at the beginning of the experiment. Gross lesions of the disease in the large intestine occurred after 80 days. Griffith (1924) obtained numerous caseous tubercles and open ulcers in the small and large intestine of guinea pigs in the remarkably short time of 44 days after the feeding of tuberculous tissue. Neither Redman nor Griffith were interested in the diets used and do not mention them. Recently (1933), McConkey and Smith showed ulcerative intestinal tuberculosis to be brought about by feeding tuberculous sputum to guinea pigs subsisting on a diet partially deficient in vitamin C.

MATERIALS AND METHODS

Animals: The guinea pig was used for this study. This animal develops scurvy readily and a chronic state of this deficiency is easily maintained. The guinea pig is also susceptible to tuberculosis. The rabbit does not develop scurvy nor does the rat. Neither does the latter

animal, when inoculated with the tubercle bacillus, give a pathological picture at all comparable to tuberculosis in man. All animals were obtained from one well-supervised farm known to breed all the stock sold.

Six animals of the same sex were kept in a cage. Large metal cages with wire-mesh floor screens over removable trays were used, this type making coprophagy difficult. The cages were soaked in 5 per cent cresol solution for 15 minutes and thoroughly scrubbed each week. The animal rooms were large, well ventilated and the temperature maintained between 70–80°F. in the winter months.

The basal diet[3]*:*

	per cent
Sobee*	76
Alacta†	20
Brewer's yeast	3
Codliver oil	1

The Sobee was autoclaved 15 minutes at 15 pounds' pressure. The Alacta was spread on trays and heated for 2 hours at 110°C. in the hot-air sterilizer. The diet was placed on trays, distilled water added to make a dough, and this mixture dried by fans. Broken cracker was fed and hay given *ad lib.* The animals received iodine in water once a week and distilled water daily. They were fed and watered daily except Sunday, extra rations being given on Saturday.

The control animals received 5 cc. of fresh orange juice 6 times a week. The chronically deficient animals were given 1 cc. of orange juice 6 times a week for the first 6 weeks, 1 cc. 5 times a week for 6 weeks and then 1 cc. 5 or 6 times a week according to the symptoms of scurvy present.

Scurvy: The guinea pigs used had initial weights of 200–600 gm., but the weights of animals in each experimental group varied little. Animals of both sexes were used in each experiment. Those receiving no orange juice developed acute scurvy and died in 3–6 weeks. Guinea pigs maintained in a chronic state of scurvy developed mild and moderate symp-

[3] The constituents of the basal diet were supplied through the courtesy of Mead Johnson and Company:

	per cent		per cent
*Soy bean flour	61	†Butter fat	12.00
Olive oil	19	Protein	33.00
Arrowroot starch	9	Lactose	46.50
Dicalcium phosphate	4	Salts	7.00
Sodium chloride	1	Moisture	1.50
Dextri-maltose	6		

toms of the deficiency. Animals receiving 5 cc. of the orange juice showed no signs of scurvy.

Tuberculin tests: All animals at the onset of the experiment gave negative skin reactions to tuberculin when injected intracutaneously with 0.1 cc. of 5 per cent Old Tuberculin. After inoculation with tubercle bacilli, both negative and positive reactions were noted and the sum of the number of pluses in the chronic vitamin-C animals and in non-scorbutic guinea pigs were compared.

Infection of animals: The Saranac Laboratory strains H37 and R1 of tubercle bacilli were used for inoculation. Weighed, normal saline suspensions of organisms grown for 2 weeks on Petroff's glycerine-egg media were used, except in feeding experiments, when heavy suspensions of H37 or sputum obtained from pulmonary tuberculosis patients were given.

Pathological procedure: A careful autopsy was performed on each animal. The scoring for tuberculosis was adapted from the method employed by Petroff and Steenken (1930) and is as follows.

1 plus: Tuberculous lesions at the site of inoculation with or without lymph-node involvement. A few isolated tubercles may be found in the lung and spleen.

2 plus: Tubercles in 2 or 3 of the organs usually involved; that is, lung, liver, spleen and lymph nodes.

3 plus: Generalized tuberculosis not of the fulminating type (see 4). In this category were also included animals with extensive active disease, such as tuberculosis pneumonia.

4 plus: Fulminating generalized tuberculosis; that is, very large spleen with infarcts, infarct in the liver, multiple tubercles in the lungs and involvement of the lymph nodes. Ruptured spleen and allergic phenomena were not uncommon in this group.

Sections were fixed in 4 per cent formalized saline solution and these were embedded in paraffin, sectioned and stained by the haematoxylin-eosin method for histological study. Sections were also examined for the presence of tubercle bacilli. These were stained for 45 minutes at 56°C. in Neelsen's carbol-fuchsin, and destained and counterstained in Gabbett's solution.

Statistical method: The weights and survival times were analyzed by statistical methods. The problem which presented itself was the following: Given two series of weights (or survival times), $x_1, x_2, x_3, x_4 \ldots, x_{n_1+1}$ and $y_1, y_2, y_4 \ldots, y_{n_2+1}$, of comparable chronic vitamin-C

deficient and control animals, to calculate the mean weights (or survival times) of each series, then to determine whether or not the difference between the two means is statistically significant. When n_1 and n_2 are greater than 30, it is only necessary to calculate the standard error (or probable error) of the difference, and if the difference between the means is greater than twice the standard error (or three times the probable error), it is considered statistically significant. Since, in the present study, however, the values of n_1 and n_2 were less than 30, a special method of analysis had to be used. (This method is given in R. A. Fisher's *Statistical Methods*, page 107, Oliver and Boyd, London (1930)):

First the means of the comparable series, $\bar{x}$ and $\bar{y}$ respectively are calculated in the usual way, by taking the sum of the absolute values and dividing by the number of cases.

Thus, in the first series:

$$\bar{x} = \frac{1}{n_1 + 1} S(x)$$

and in the second series:

$$\bar{y} = \frac{1}{n_2 + 1} S(x)$$

The difference between the means is $\bar{x} - \bar{y}$. Then, in place of the standard error of the difference, a statistic

$$s\sqrt{\frac{1}{n_1 + 1} + \frac{1}{n_1 + 1}},$$

which may more conveniently be designated simply as $\bar{s}$, is calculated by means of the following formula:

$$s^2 \frac{1}{n_1 + 1} \frac{1}{n_2 + 1} \text{ or } \bar{s}^2 = \frac{(n_1 + n_2 + 2)}{(n_1 + 1)(n_2 + 1)(n_1 + n_2)} S(x - \bar{x})^2 + S(y - \bar{y})^2$$

where $S(x - \bar{x})^2$ and $S(y - \bar{y})^2$ represent the sum of the squares of the deviations of the observed weights (or survival times) in the respective series. Therefore, $\bar{s}$, like the standard error of the difference, is a measure of the statistical error of the difference between the means.

The statistical significance of the difference therefore depends upon

$$t = \frac{\bar{x} - \bar{y}}{\bar{s}}$$

When $n = n_1 + n_2$ is greater than 30, this reduces to the usual formula, but when $n = 30$, a statistic P is derived by consulting the table in Fisher's book (p. 139). P merely gives the frequency with which a value of t greater than that observed could result from chance alone. The convention is to consider the difference between the means significant when $P < .05$.

Example: To calculate the significance of the difference between the mean weights at 10 weeks of chronic vitamin-C deficient and control animals fed strain H37 (table 15). If the former series be designated by $x_1, x_2 \ldots x_{n_1+1}$ and the latter by $y_1, y_2 \ldots y_{n_2+1}$, then

$$n_1 = 16$$

$$n_2 = 16$$

$$\bar{x} = \frac{S(x)}{17} = 386$$

$$\bar{y} = \frac{S(x)}{17} = 455$$

$$\bar{x} - \bar{y} = 69$$

$$S(x - \bar{x})^2 = 21{,}627$$

$$X(y - \bar{y})^2 = 37{,}047$$

Hence, $$\bar{s}^2 = \frac{(16 + 16 + 2)\ (21{,}627 + 37{,}047)}{17 \times 17 \times 32} = 215.7132$$

$$\bar{s} = 14.69$$

$$t = \frac{69}{14.69} = 4.7$$

$$n = n_1 + n_2 = 32$$

Hence, $P < 0.01$. So the difference between the means is statistically significant.

PROTOCOLS OF THE EXPERIMENTS

Group 1: Animals used to control the diets: Twelve guinea pigs on the partially deficient vitamin-C diet and 12 animals on an ample diet were used.

Pathology: Three partially depleted vitamin-C animals and 1 non-

TABLE 1

The weights and survival period of uninoculated animals

ANIMAL NUMBER	WEIGHT IN GRAMS AT							SURVIVAL (DAYS)
	Onset	6 weeks	12 weeks	18 weeks	24 weeks	30 weeks	Death	
Experimental*								
58-1	242	348	425	460	465	575	635	221
58-2	285	310	390	440	465	515	540	221
58-3	275	312	387	490	495	535	585	221
58-4	335	337	380	445	380	355	340	221
58-5	295	312	403	505	525	595	585	221
58-6	298	390	445	388	460	520	530	221
59-1†	318	405	395	370	385	—	380	198
59-2	328	418	435	490	475	545	545	220
59-3†	298	463	480	555	535	—	365	206
59-4	322	445	445	500	510	505	525	220
59-5†	288	360	430	550	640	—	610	197
59-6	300	425	438	510	505	565	620	220
Control**								
57-1	265	398	450	540	618	635	690	221
57-2	290	345	375	488	540	605	650	221
57-3	345	452	472	518	570	605	640	221
57-4	290	412	435	500	550	615	665	221
57-5	385	415	445	468	480	570	625	221
57-6	330	348	415	475	435	425	445	221
60-1	327	440	455	540	575	610	615	220
60-2†	305	415	435	435	—	—	395	155
60-3	290	412	408	498	560	650	690	220
60-4	276	290	302	400	495	575	610	220
60-5	202	378	352	415	425	470	500	220
60-6	318	418	405	440	485	540	585	220
Experimental mean....	299	377	421	475	487	527	521	205
Control mean.....	302	394	412	476	521	573	593	196
Difference.....	3	17	9	1	34	46	72	—
t.....	0.262	0.194	0.67	0.047	0.751	0.676	1.77	—
n.....	22	22	22	22	21	19	22	—
P.....	0.79	0.84	0.51	0.9	0.21	0.14	0.058	—

* Guinea pigs subsisting on a diet partially deficient in vitamin C.

† Died; the other animals were killed.

** Nonscorbutic animals.

scorbutic guinea pig died before the 31st week. The surviving animals were then killed (table 1). No significant difference in weights was found until near the termination of the experiment, when the difference

was beginning to be significant (table 1 and figure 1). However, as is seen in the scurvy score (table 2), the animals receiving the diet poor in vitamin C showed clinical symptoms of scurvy: swelling of the joints, lameness, looseness of the teeth, for many weeks before the group was killed. At autopsy, additional scorbutic lesions were present.

Tuberculin tests were done 28, 57, 112 and 191 days, respectively, after the beginning of the diet to rule out accidental infection. Animal 59–5 gave a 1-plus reaction at the last test and at autopsy a very few minute areas of early tuberculosis were found in the spleen and lung.

TABLE 2

Scurvy findings in uninoculated animals

ANIMAL NUMBER	CLINICAL SYMPTOMS	BONY SYSTEM			HAEMORRHAGES				SCORE
		Teeth	Ribs	Joints	Ribs	Intestines	Joints	Muscle	
Experimental									
58-1	mild	1	½	0	0	0	2	2	5½
58-2	moderate	0	0	1	2	0	2	4	9
58-3	mild	1	0	1	0	0	2	4	8
58-4	moderate	2	0	0	0	0	2	2	6
58-5	moderate	0	2	0	0	1	1	3	7
58-6	moderate	1	0	0	0	1	0	4	6
59-1	mild	2	2	0	1	0	1	3	9
59-2	mild	1	0	2	0	0	1	2	6
59-3	negative	3	2	0	0	0	0	2	7
59-4	negative	1	1	1	0	0	2	4	9
59-5	mild	1	0	2	0	0	1	3	7
59-6	negative	0	0	0	0	0	1	4	5
Control	negative	0	0	0	0	0	0	0	0

The other animals gave negative skin reactions and at autopsy showed no gross lesions of tuberculosis.

Guinea pigs were maintained in a condition of chronic scurvy for 31 weeks. One animal developed spontaneous tuberculosis.

Group 2: Infection produced by intraperitoneal inoculation: Twenty-four guinea pigs were inoculated intraperitoneally with 0.157 mgm. of tubercle bacilli, H37. They were then divided into two groups, one of which received the diet partially deficient in vitamin C and the other that containing ample orange juice.

Pathology: The survival period and weights of the two groups were not significantly different, as is shown in table 3 and figure 2. Practically

TABLE 3

The weights and survival time of guinea pigs inoculated intraperitoneally

ANIMAL NUMBER	WEIGHT IN GRAMS AT				SURVIVAL IN (DAYS)
	Onset	6 weeks	10 weeks	Death	
Experimental					
1-1	370	355	—	282	56
1-2	360	345	—	322	55
1-3	380	377	292	280	76
1-4	375	320	287	272	75
1-5	340	397	327	327	69
1-6	355	350	—	275	65
2-1	348	352	325	292	56
2-2	350	330	242	242	70
2-3	350	315	—	332	46
2-4	341	247	—	247	40
2-5	355	375	360	237	92
2-6	360	395	357	272	52
Control					
11-1	365	240	—	240	42
11-2	375	275	—	252	45
11-3	390	365	—	332	57
11-4	380	415	447	378	100
11-5	375	365	357	304	87
11-6	365	375	365	252	91
12-1	347	290	—	250	55
12-2	365	347	362	232	115
12-3	370	385	377	287	88
12-4	360	325	—	275	49
12-5*	295	425	497	588	137
12-6	340	330	267	270	75
Chronic mean.....	357	346	313	282	62
Control mean.....	360	345	382	302	78
Difference.....	3	1	69	20	16
t.....	0.237	0.049	0.922	0.666	1.68
n.....	22	22	12	22	22
P.....	0.81	>0.9	0.053	0.41	0.11

* Killed.

all the animals developed generalized tuberculosis (table 4). The total score was about the same in both groups; 12 of the chronic scorbutic animals had 3-plus or more involvement as against 10-plus of the con-

trol group. There seemed to be slightly more severe involvement of the spleen in the control animals while there was a slightly greater amount of tuberculosis in the lungs in the scorbutic group. Tubercle

TABLE 4

Macroscopic pathology on intraperitoneally inoculated animals

ANIMAL NUMBER	LUNG	LIVER	SPLEEN	LYMPH NODES					TB. SCORE	SCURVY SCORE	SURVIVAL
				Ing	R-p	T-b	Hep	Mes			
Experimental											
1-1	3+	1+	1+	1*	2	—	1	1	3+	2	56
1-2	3+	0	1+	0	1	1	1	1	3+	2	55
1-3	4+	?	1+	1	1	2	1	—	3+	5	76
1-4	4+	1+	1+	0	0	1	1	—	3+	2	75
1-5	4+	?	1+	1*	—	1	1	—	3+	¼	69
1-6	4+	1+	1+	1	2	1	2	2	3+	¼	65
2-1	4+	4+	1+	1*	1*	2	—	0	3+	3	56
2-2	3+	1+	2+	1	1	1	1	—	3+	1½	70
2-3	4+	3+	3+	1	2	2	2	1	4+	¾	46
2-4	3+	3+	3+	1	2	3	2	2	3+	4	40
2-5	3+	3+	1+	1*	2**	1	2	2	3+	4	92
2-6	1+	2+	4+	3*	1*	1	?	2	3+	7	82
Total	40	19	20	12	15	16	14	11	37	27½	—
Control											
11-1	3+	3+	3+	1	1	2	3	2*	3+	0	42
11-2	2+	3+	3+	1	1	2	2	1	3+	0	45
11-3	4+	1+	2+	2	1	2	1	1	3+	0	57
11-4	3+	1+	4+	1	—	1	1	3**	3+	0	70
11-5	4+	2+	3+	1	1	1	1	—	3+	0	87
11-6	3+	0	2+	2**	2**	2**	2	4**	3+	0	91
12-1	3+	3+	3+	1	1	2	2	2	4+	0	55
12-2	3+	0	1+	1	1	1	1	3*	3+	0	115
12-3	4+	1+	3+	1	1	3	2	2**	3+	0	88
12-4	3+	2+	1+	1	1	1	2	1	3+	0	49
12-5†	2+	1+	1+	2*	2	4*	3	2*	2+	0	137
12-6	2+	1+	3+	1*	1	2	0	0	3+	0	75
Total	36	18	29	15	13	23	20	21	36	0	—

* Caseation.
** Calcification.
† Killed.

bacilli were found in practically all the organs examined. Microscopically (table 5), there was no difference in the pathological findings in both series.

TABLE 5

Microscopic pathology on guinea pigs inoculated intraperitoneally

ANIMAL NUMBER	ORGANS	HAEMORRHAGE	INFILTRATION	NECROSIS	ANIMAL NUMBER	ORGANS	HAEMORRHAGE	INFILTRATION	NECROSIS
Experimental**					Control				
1-3	Lung*	0	3+	0	11-2	Lung*	0	2	1+
	Liver*	2+	3+	2		Liver*	3+	4+	1+
	Spleen*	2+	3+	1+		Spleen*	0	3+	3+
1-4	Lung*	1+	3+	0	11-3	Lung*	1+	3+	0
	Liver*	1+	1+	1+		Liver*	3+	1+	0
	Spleen*†	1+	2+	4+		Spleen*	2+	3+	2+
1-5	Lung*	1+	2+	3+	11-4	Lung*	0	3+	0
	Liver*	1+	0	2+		Liver*	1+	3+	3+
	Spleen*	1+	4+	2+		Spleen*†	1+	4+	2+
1-6	Lung*	1+	3+	0	11-5	Lung*	1+	3+	1+
	Liver*	1+	3+	0		Liver*†	3+	3+	2+
	Spleen*	2+	3+	2+		Spleen*†	3+	4+	4+
2-1	Lung*	0	3+	2+	11-6	Lung*	0	3+	1+
	Liver*	0	3+	2+		Liver*	3+	3+	0
	Spleen*	0	3+	1+		Spleen*	1+	2+	3+
2-2	Lung*	1+	2+	1+	12-1	Lung*	0	2+	0
	Liver	2+	3+	2+		Liver*	2+	2+	3+
	Spleen*†	1+	3+	1+		Spleen*	1+	2+	2+
2-3	Lung*	1+	2+	0	12-2	Lung*	0	4+	1+
	Liver	3+	2+	3+		Liver*	0	4+	1+
	Spleen*	1+	3+	3+		Spleen*	0	2+	1+
2-4	Lung*	2+	2+	0	12-3	Lung*	1+	2+	1+
	Liver*	2+	3+	0		Liver*	1+	4+	2+
	Spleen*†	2+	4+	0		Spleen*	4+	3+	3+
2-5	Lung*	1+	2+	1+	12-4	Lung*	0	2+	1+
	Liver*	1+	2+	2+		Liver*	2+	2+	3+
	Spleen*	0	3+	2+		Spleen*†	0	3+	1+
2-6	Lung	2+	3+	2+	12-5	Lung*	0	3+	2+
	Liver	0	2+	2+		Liver	0	1+	0
	Spleen	0	3+	3+		Spleen	0	2+	2+
Control					12-6	Lung*	0	2+	0
11-1	Lung*	0	2+	1+		Liver*†	2+	3+	3+
	Liver*	2+	3+	2+		Spleen*	2+	2+	4+
	Spleen*	2+	3+	2+					

* Tubercle bacilli found.

** Sections on 1-1 and 1-2 unsatisfactory.

† Calcification.

The partial depletion of vitamin C did not result in pathological changes differing significantly from those in the control animals.

TABLE 6

Macroscopic pathology on animals inoculated subcutaneously with 0.1 mgm. of H37

GUINEA PIG NUMBER	LUNG	LIVER	SPLEEN	LYMPH NODES					TB. SCORE	SURVIVAL	SCURVY SCORE
				Ing	R-p	T-b	Hep	Mes			
Experimental											
3-1	3+	1+	1+	1*	2	3	0	1	3+	61	½
3-2	4+	3+	4+	2**	2**	1	3**	2**	4+	133	7
3-3	2+	3+	4+	3**	3*	3**	4**	3*	4+	80	0
3-4	3+	4+	4+	4*	3*	1*	2*	—	4+	79	7
3-5	2+	?	2+	1*	1	2	2	2	3+	61	0
4-1	2+	4+	4+	2*	2	0	—	2	3+	53	0
4-2	2+	3+	3+	1**	0	1**	2**	1**	4+	97	2½
4-3	3+	3+	4+	2*	3*	3*	2	2*	4+	61	0
4-4	2+	3+	3+	2**	1**	1**	1	2**	4+	96	3
4-5	1+	4+	4+	2*	2*	0	2*	0	3+	77	2½
5-1	3+	2+	2+	2	1	3	3	2*	4+	67	1
5-2	3+	2+	4+	2*	3*	3*	2*	2*	3+	70	0
5-3	1+	0	0	1*	0	0	1	1	1+	150	10
5-4	2+	2+	4+	1	2	2	2	2	4+	121	2½
5-5	2+	3+	3+	3	3	2	3	1	3+	52	0
Total	35	37	46	29	28	25	29	23	51	—	36
Control											
13-1	2+	1+	2+	1	1	1	1	2	4+	127	0
13-2	3+	2+	4+	1**	2*	1	3*	1	3+	156	0
13-3	3+	4+	4+	3**	3**	3**	2**	1**	4+	92	0
13-4	3+	2+	1+	1	1	3**	3**	3**	3	156	0
13-5	4+	4+	3+	4*	3*	4*	4	4*	4+	85	0
14-1	1+	?	1+	2**	3**	2**	2**	2**	1+	157	0
14-2	3+	1+	2+	2	2	3	3	2	3+	61	0
14-3	3+	3+	3+	2*	0	2*	2**	2*	4+	149	0
14-4	4+	2+	3+	1**	1*	3	3**	3**	3+	157	0
14-5	2+	4+	4+	2**	2**	1**	2	2**	4+	97	0
15-1	1+	?	1+	1*	0	—	—	0	1+	67	0
15-2	3+	3+	3+	1*	1	2**	3**	3**	3+	158	0
15-3	1+	4+	4+	1*	2**	1**	2	—	4+	152	0
15-4	4+	?	3+	1*	1	2	2	1	3+	67	0
15-5	3+	4+	4+	1*	1	—	—	3*	4+	152	0
15-6	0	3+	4+	1*	2*	1**	1	3*	3+	91	0
Total	40	37	46	25	25	29	33	32	51	—	0

* Caseation.

** Calcification.

Group 3: Animals inoculated subcutaneously with culture H37: These guinea pigs were infected by subcutaneous inoculation with tubercle bacilli, H37, and immediately placed on the chronic scurvy producing and control diets.

A: The infecting dose for 31 animals was 0.1 mgm.

Pathology: Both nonscorbutic and chronic scorbutic animals had generalized involvement. Although there was no difference in the macroscopic findings (table 6), the survival time was significantly different in both groups (control mean = 133 days, experimental mean = 91 days, P = .01) as is seen in table 7. Thus, the animals on the partially deficient diet had generalized tuberculosis of the same extent as was found at a later period in control guinea pigs. Microscopically, there was no difference in the character of the lesions in the comparable groups (table 8). In the chronic scorbutic group, 77 per cent of all the sections examined were positive for tubercle bacilli in contrast to 63 per cent in the control sections.

Table 7 presents the weights and figure 3 gives a graphic picture of the weight-curve. The guinea pigs on the vitamin-C deficient diet had, after 6 weeks, weights significantly lower than the animals on the ample diet. Tuberculin tests showed no significant difference in the skin sensitivity of the partially deficient and control groups two weeks after inoculation and at the beginning of the diet.

B: Twelve guinea pigs were placed on the chronic scorbutic and 9 on the nonscorbutic diets; at the same time, they were inoculated with 0.012 mgm. of the H37 strain of tubercle bacillus.

Pathology: The weights, which are shown in table 9, were not significantly different. The experimental animals had a survival mean of 54 days and the controls one of 84 days; this closely approached a significant value as P = .022. It should be noted that the animals in this experiment weighed, on an average, 100 gm. less than those in group 3a. Generalized tuberculosis was present in 5 of 12 of the partially depleted animals and in 8 of 9 of the controls (table 10). A less extensive involvement with tuberculosis than that found in nonscorbutic guinea pigs apparently caused the death of young animals when they were on a diet producing chronic scurvy. The survival period was not long enough for the lack of the vitamin to affect the tuberculous process. Although the infecting dose was smaller, the age factor probably accounts for the difference in the survival period of the animals in this experiment and those in group 3a.

The sensitivity to the tuberculin test did not differ in the chronic vitamin-C depleted and control animals when they were tested two weeks after inoculation. Both gave negative or 1 plus reactions.

TABLE 7

The weights and survival time of animals inoculated subcutaneously with 0.1 mgm. H37

ANIMAL NUMBER	WEIGHT IN GRAMS AT					SURVIVAL IN DAYS
	Onset	6 weeks	10 weeks	12 weeks	Death	
Experimental						
3-1*	345	375	—	—	256	61
3-2	365	442	420	342	342	133
3-3	355	370	367	—	335	80
3-4	337	320	307	—	272	79
3-5*	290	337	—	—	322	61
4-1	330	300	—	—	255	53
4-2	340	390	425	295	270	97
4-3*	335	330	—	—	318	61
4-4	340	415	405	335	257	96
4-5	325	372	282	—	262	77
5-1*	405	385	—	—	310	67
5-2	395	295	327	—	327	70
5-3	375	425	462	462	350	150
5-4	385	430	437	430	450	121
5-5	370	305	—	—	255	52
Control						
13-1	390	390	380	420	310	127
13-2**	385	432	448	437	425	156
13-3	367	430	380	297	302	92
13-4**	390	455	457	442	447	156
13-5	385	455	400	—	352	85
14-1**	370	390	425	407	422	157
14-2*	352	362	—	—	370	61
14-3	385	420	457	427	332	149
14-4**	390	495	523	520	542	157
14-5	330	435	447	415	327	97
15-1*	345	417	—	—	440	67
15-2**	363	390	475	497	477	158
15-3	375	430	415	385	312	152
15-4*	345	340	—	—	315	67
15-5	365	392	440	457	440	152
15-6	365	505	482	380	387	91
Chronic mean.....	352	366	381	373	305	91
Control mean.....	368	421	441	424	362	133
Difference.....	16	55	60	51	57	42
t.....	1.67	3.32	2.68	1.55	2.12	3.94
n.....	29	29	20	15	29	23
P.....	0.11	<0.01	0.015	0.14	0.028	<0.01

* Died following bleeding from heart.
** Killed.

TABLE 8

Microscopic pathology of animals inoculated subcutaneously with 0.1 mgm. H37

ANIMAL NUMBER	ORGANS	HAEMORRHAGE	INFILTRATION	NECROSIS	CALCIFICATION	ANIMAL NUMBER	ORGANS	HAEMORRHAGE	INFILTRATION	NECROSIS	CALCIFICATION
Experimental						Control					
3-1	Lung*	0	1+	0	0	13-1	Lung*	0	2+	0	0
	Liver*	0	2	0	1+		Liver*	0	2+	1+	0
	Spleen*	3+	1+	4+	1+		Spleen*	0	1+	4+	1+
3-2	Lung*	1+	4+	2+	2+	13-2	Lung*	1+	2+	1+	0
	Liver*	1+	3+	2+	1+		Liver*	1+	3+	2+	0
	Spleen*	0	3+	4+	1+		Spleen*	1+	3+	3+	0
3-3	Lung	0	1+	0	1+	13-3	Lung	0	2+	2+	0
	Liver*	1+	1+	2+	2+		Liver	0	1+	2+	0
	Spleen*	0	0	4+	2+		Spleen*	0	2+	4+	0
3-4	Lung*	0	1+	1+	0	13-4	Lung*	0	1+	1+	0
	Liver*	0	2+	2+	0		Liver	0	1+	1+	0
	Spleen*	2+	2+	4+	3+		Spleen	2+	3+	1+	0
3-5	Lung*	0	1+	0	0	13-5	Lung	—	—	—	—
	Liver*	0	2+	2	0		Liver*	0	2+	2+	1+
	Spleen*	3+	2+	3+	0		Spleen*	1+	2+	3+	0
4-1	Lung*	0	3+	0	0	14-1	Lung	1	1+	0	0
	Liver*	0	2+	0	0		Liver	2+	2+	2+	0
	Spleen*	1+	3+	3+	0		Spleen*	1+	2+	3+	?
4-2	Lung	0	1+	0	0	14-2	Lung*	1+	2+	0	0
	Liver*	1+	2+	2+	0		Liver	1+	3+	1+	0
	Spleen*	0	1+	0	0		Spleen	1+	3+	1+	0
4-3	Lung	0	4+	0	0	14-3	Lung*	1+	3+	2+	0
	Liver	0	3+	2+	0		Liver*	2+	3+	2+	0
	Spleen	0	3+	3+	0		Spleen*	2+	2+	4+	0
4-4	Lung*	0	2+	0	0	14-4	Lung*	0	4+	1+	0
	Liver	0	1+	2+	0		Liver	0	1+	2+	0
	Spleen	4+	1+	4+	0		Spleen	1	2+	2+	2+
4-5	Lung	0	0	0	0	14-5	Lung*	0	1+	0	0
	Liver*	0	2+	1+	0		Liver*	0	2+	2+	0
	Spleen*	0	0	3+	0		Spleen*	0	2+	4+	0
5-1	Lung*	0	2+	3+	0	15-1	Lung*	0	1+	0	0
	Liver*	0	2+	1+	0		Liver	0	0	0	0
	Spleen*	1+	2+	1+	0		Spleen	0	2+	3+	1+
5-2	Lung*	0	2+	3+	0	15-2	Lung*	1+	3+	0	0
	Liver*	0	1+	0	0		Liver	0	2+	0	0
	Spleen*	0	3+	0	0		Spleen	1+	3+	3+	0
5-4	Lung	0	1+	0	0	15-3	Lung*	0	1+	0	0
	Liver	0	1+	0	0		Liver*	0	2+	3+	0
	Spleen*	0	3+	2+	0		Spleen*	0	2+	2+	0
5-5	Lung*	0	2+	0	0	15-4	Lung*	0	4+	2+	0
	Liver*	0	1+	0	0		Liver	0	2+	0	0
	Spleen*	0	2+	2+	0		Spleen*	0	2+	3+	?
						15-5	Lung*	1+	4+	3+	0
							Liver	0	3+	3+	0
							Spleen	0	0	4+	0

5-3 sections not satisfactory.

* Tubercle bacilli found.

Thirty-seven days after the inoculation, the score for the deficient vitamin C group of 10 animals was 13 as contrasted to that of 7 control animals who gave a tuberculin score of 21. Two deficient and 1 control

TABLE 9

The weights and survival time of animals inoculated subcutaneously with 0.012 mgm. H37

ANIMAL NUMBER	WEIGHTS IN GRAMS AT			SURVIVAL IN DAYS
	Onset	6 weeks	Death	
Experimental				
36-1	213	335	210	75
36-2	230	—	263	36
36-3	247	195	172	46
37-1	270	265	278	78
37-2	217	205	205	44
37-3	245	237	237	41
38-1	275	—	200	37
38-2	220	198	180	44
38-3	290	275	188	97
39-1	270	217	185	46
39-2	248	178	178	59
39-3	265	225	230	51
Control				
32-1	240	248	297	119
33-1	238	—	187	35
33-2	240	277	225	66
33-3	220	225	195	49
34-1	270	292	220	66
34-2*	245	290	475	142
34-3	268	218	198	129
35-2	223	260	233	67
35-3	265	240	245	87
Chronic mean.....	249	233	210	54
Control mean.....	245	256	253	84
Difference.....	4	23	43	30
t.....	3.97	1.24	1.52	2.43
n.....	19	16	19	19
P.....	0.69	0.23	0.14	0.022

* Killed.

guinea pigs died before the test was read. When tested at 80 days, the 3 control animals gave a score of 7 and the 1 remaining scorbutic animal that of 3.

Animals inoculated subcutaneously with strain H37 and placed on a diet partially depleted in vitamin C showed a greater loss of body weight and a shorter survival period than did the control animals. Generalized tuberculosis developed more rapidly than in the control animals. When

TABLE 10

The macroscopic findings at autopsy on animals inoculated subcutaneously with 0.012 mgm of H37

ANIMAL NUMBER	LUNG	LIVER	SPLEEN	LYMPH NODES					INTESTINE	TB. SCORE	SCURVY SCORE	SURVIVAL
				Ing	R-p	T-b	Hep	Mes				
Experimental												
36-1	2+	2+	1+	1*	1	0	0	1	0	2+	2	75
36-2	1+	1+	1+	3*	3*	3	1*	1	0	2+	5	36
36-3	cons. tb.?	2+	2+	1*	1	1	0	1	0	2+	5	46
37-1	3+	2+	3+	2*	1	1	0	1	0	3+	1½	78
37-2	0	1+	3+	3*	2*	2	—	2*	0	3+	5	44
37-3	0	0	2+	2	2*	0	0	0	0	2+	2	41
38-1	0	?	0	1*	1*	1	0	1	0	1+	2	37
38-2	0	2+	1+	2*	1*	0	0	0	0	2+	3	44
38-3	4+	4+	2+	2*	1*	0	1*	1*	0	4+	1	97
39-1	cons. tb.?	0	2+	1*	1*	2*	—	1	0	2+	1	46
39-2	1+	3+	3+	1*	2*	1*	1*	2*	0	3+	0	59
39-3	1+	4+	3+†	2*	2*	1	2*	1	0	4+	1	51
Control												
32-1	3+	2+	2+	2**	1	2*	0	2*	colon?	3+	0	119
33-1	cons.	0	0	1	0	0	0	0	0	1+	0	35
33-2	1+; pl.	2+	2+	2*	1	0	0	0	0	3+	0	66
33-3	2+	4+	2+	2*	1*	0	0	1*	0	4+	0	49
34-1	3+	1+	2+	1*	1*	0	0	0	0	3+	0	66
34-2	3+	4+	3+	3*	2	3	0	2	0	4+	0	142
34-3	3+; pl.	3+	1+	3*	2*	2*	—	3*	0	3+	0	129
35-2	cons. 3+	3+	3+	4*	3*	4*	3*	3*	0	3+	0	67
35-3	3+	2+	3+‡	2*	1*	1	0	1	0	4+	0	87

* Caseation.
** Calcification.
† Killed.
‡ Infarct. Cons. = consolidation. Pl. = pleurisy.

young animals were used, these differences were not apparent but the guinea pigs on the diet deficient in vitamin C tended to have a decreased skin sensitivity to tuberculin.

Group 4: Tuberculous infection and acute scurvy: These animals were used to test the effect of acute scurvy on the progress of tuberculosis. Twenty-

three guinea pigs were inoculated subcutaneously with 0.1 mgm. of strain H37. Two weeks later they were divided into two groups, one receiving the basal diet and the other the control amount of vitamin C, 5 cc. of orange juice given 6 times weekly.

Pathology: The animals placed on the deficient diet were dead within 36 days. All control animals were then killed. Acute scurvy caused a decidedly significant difference in weights as was to be expected (table 11). There appeared to be more involvement in the lungs in the control

TABLE 11

The weights and survival time of acute scurvy and control animals inoculated subcutaneously with 0.1 mgm. H37

ANIMAL NUMBER ACUTE SCURVY	WEIGHT AT INOCULATION	WEIGHT AT DEATH	SURVIVAL IN DAYS	ANIMAL NUMBER: CONTROL	WEIGHT AT INOCULATION	WEIGHT AT DEATH	SURVIVAL IN DAYS
24-1	360	237	43	26-1*	328	360	47
24-2	332	315	42	26-2*	352	348	47
24-3	337	228	41	26-3*	328	450	49
24-4	327	272	42	26-4*	255	352	52
24-5	335	292	45	26-5*	308	367	51
24-6	267	177	41	26-6*	285	407	51
25-1	310	220	41	27-1	360	272	35
25-2	330	235	50	27-2*	352	427	48
25-4	355	262	44	27-3*	372	310	49
25-5	340	257	46	27-4*	297	292	47
25-6	340	230	45	27-5*	342	315	49
				27-6*	284	245	49
Acute mean.....	330	267	42	t.....	0.61	3.41	—
Control mean.....	322	345	48	n.....	21	21	—
Difference.....	8	78	6	P.....	0.54	<0.01	—

* Killed.

group, although the scores for generalized tuberculosis were practically the same (table 12). Generalized tuberculosis developed in both groups and there was no difference in the tuberculin tests.

Apparently the complete deficiency of vitamin C was without affect on the tuberculous process.

Group 5: Infection with avirulent R1: Twelve animals were placed on the chronic scorbutic diet and 12 on the complete diet and inoculated subcutaneously with 0.96 mgm. of strain R1.

Pathology: The experiment terminated at 28 weeks, when the surviving animals, 8 experimental and 11 controls, were killed (table 13). On comparing the groups at 16 and 24 weeks and at death, it will be noted

TABLE 12

Macroscopic pathology of acute scurvy and control animals inoculated subcutaneously with 0.1 mgm. of H37

ANIMAL NUMBER	LUNG	LIVER	SPLEEN	LYMPH NODES						TB. SCORE	SCURVY SCORE
				Ing	R-p	T-b	Hep	Mes	Cer		
Experimental											
24-1	1+	3+	2+	2*	2*	2*	3*	2*	0	3+	14
24-2	1+	2+	1+	3*	2*	3*	2*	1*	2*	3+	13
24-3	1+	2+‡	2+	2*	2*	3*	4*	3*	0	4+	4
24-4	1+	2+	2+	2*	1*	2*	2*	2*	0	3+	14
24-5	1+	4+	1+	2*	2*	3*	—	2*	2*	3+	7
24-6	0	1+?	1+	2*	1*	2*	3*	1*	3*	1+	8
25-1	2+	2+	2+	2*	2*	2*	3*	—	0	3+	2
25-2	1+	2+	2+	3*	2*	3*	1*	1*	2*	3+	8
25-4	1+	3+	2+	1*	1	3*	2*	3*	2*	3+	8
25-5	3+	2+‡	1+	2*	3*	3*	—	2*	1*	4+	8
25-6	0	2+	2+	2*	1*	3*	1*	2*	1*	3+	5
Total	12	25	18	23	19	29	21	19	13	33+	—
Control†											
26-1	3+	2+	1+	1*	1*	2*	3*	1	1	3+	0
26-2	3+	1+	2+	2	2*	2	3*	3*	1	1+	0
26-3	2+	2+	2+	2*	2	3*	3*	2	3*	3+	0
26-4	2+	2+	2+	2*	3*	3*	2	1	2	3+	0
26-5	3+	1+	2+	1	2*	2*	2	1*	1	3+	0
26-6	2+	1+	2+	2*	2*	4**	3*	1	1	3+	0
27-1	3+	4+‡	4+‡	3*	2*	2*	3*	1*	3*	4+	0
27-2	2+	1+	3+	1	1*	2*	3*	1	1*	3+	0
27-3	3+	1+	1+	1*	1*	—	2*	1	1	3+	0
27-4	2+	2+‡	2+	1*	1*	3*	2*	1	1*	4+	0
27-5	4+	3+	2+	2*	3*	2*	0	1*	1*	3+	0
27-6	2+	1+?	1+	2*	2*	3*	3**	1	1*	2+	0
Total	31	21	23	20	22	28	29	15	17	35	0

* Caseation.
** And calcification.
† All killed except 27-1.
‡ Infarct.

that the weight difference was decidedly significant, as is seen in the table just mentioned and also in figure 4.

The comparable groups did not appear to differ significantly in their

TABLE 13

The weights and survival period of animals inoculated with tubercle bacilli, R1, subcutaneously

ANIMAL NUMBER	WEIGHTS IN GRAMS					SURVIVAL (DAYS)
	Onset	8 weeks	16 weeks	24 weeks	Death	
Experimental						
9-1*	370	405	322	345	330	198
9-2*	357	455	352	368	360	198
9-3*	365	405	372	320	385	198
9-4*	362	387	347	412	340	195
9-5*	385	432	385	350	355	195
9-6*	392	500	472	400	300	195
10-1	387	440	410	—	277	139
10-2	375	352	372	370	235	184
10-3*	332	282	257	357	395	197
10-4	355	385	398	422	422	175
10-5	380	400	395	378	255	181
10-6*	252	357	377	438	285	196
Control						
19-1*	322	437	508	575	580	197
19-2*	385	378	585	595	575	195
19-3*	355	430	520	555	575	197
19-4*	370	408	458	535	480	194
19-5*	355	508	632	685	687	196
19-6*	375	400	397	360	278	194
20-1*	355	460	495	625	650	199
20-2*	337	447	437	560	635	200
20-3*	370	397	345	540	528	196
20-4	247	360	—	—	257	98
20-5*	355	440	520	635	660	199
20-6*	350	395	380	500	495	200
Experimental mean....	359	400	340	379	328	187
Control mean.....	438	430	480	560	533	189
Difference.....	11	30	139	181	205	—
t.....	0.718	1.44	4.47	6.56	4.69	—
n.....	22	22	21	20	22	—
P.....	0.48	0.17	<0.01	<0.01	<0.01	—

* Killed.

macroscopic pathology. In the chronic scorbutic group there were 5 animals with small areas of calcification at the site of inoculation, 2 with semicaseous areas, and 5 with no evidence of tuberculous lesions. In

the nonscorbutic series there were 3 animals with small calcified masses at the site of inoculation, 2 with small caseous masses, and 7 with no lesion. There was no splenic enlargement in any of the animals and one deficient animal showed minimal tuberculosis of this organ. Six of the partially depleted animals occasionally showed a gelatinous subpleural area of small pin-head size in the lungs but no extensive progressive lesion. There were only 2 animals with similar areas in the control group. There were no significant changes in the lymph nodes.

Microscopically, the lesion observed was only that of mononuclear proliferation, occasionally in the spleen and lung. There was no progressive tuberculosis. Six of the chronic scorbutic animals showed very small areas of mononuclear infiltration in the lymphoid tissue of the spleen. Two of the control animals had similar pathological processes. In the lung 2 animals in the deficient group showed small areas of mononuclear infiltration in the lymphoid tissue, while in the control group 3 animals had hyperplasia of the lymphoid tissue. The sections were examined for tubercle bacilli and were negative.

TABLE 14

Tuberculin tests performed on animals inoculated subcutaneously with R1 tubercle bacillus

ANIMAL NUMBER	DAYS AFTER INOCULATION				ANIMAL NUMBER	DAYS AFTER INOCULATION			
	14	44	87	144		14	44	87	144
Experimental					Control				
9-1	1	4	1	?	19-1	1	4	3	2
9-2	1	3	1	0	19-2	1	4	2	0
9-3	?	4	?	0	19-3	1	2	2	1
9-4	2	4	1	?	19-4	1	3	1	?
9-5	1	?	2	1	19-5	1	3	3	2
9-6	?	4	1	0	19-6	2	4	2	?
10-1	0	3	2	—	20-1	1	3	2	2
10-2	?	2	2	0	20-2	1	3	1	?
10-3	2	2	3	0	20-3	1	2	1	3
10-4	0	2	3	0	20-4	1	3	—	—
10-5	?	2	?	0	20-5	1	4	4	2
10-6	1	2	3	0	20-6	1	2	2	?
Score	11	32½	20	2	Score	13	37	23	14

The tuberculin tests are shown in table 14. It will be noted that the intensity of the reactions was slightly less in the chronic scorbutic animals, particularly at 144 days after inoculation with tubercle bacillus, R1.

A decrease in weight and dermal sensitivity to tuberculin occurred in animals inoculated with strain R1, and maintained in a state of chronic scurvy. However, there was no significant difference in tuberculosis as compared to that in the control animals.

TABLE 15

The weights and survival period of guinea pigs fed H37

ANIMAL NUMBER	ONSET	10 WEEKS	20 WEEKS	AT DEATH	SURVIVAL	ANIMAL NUMBER	ONSET	10 WEEKS	20 WEEKS	AT DEATH	SURVIVAL
Experimental						Control					
6-1	380	382	—	225	138	16-1	380	420	547	525	161*
6-2	342	400	330	298	178*	16-2	360	440	518	540	162*
6-3	362	310	—	287	85	16-3	360	435	425	530	159*
6-4	362	380	—	362	108	16-4	337	535	628	647	159*
6-5	315	340	322	310	161	16-5	345	402	—	335	113*
6-6	360	397	—	252	125	16-6	360	440	595	645	161*
7-1	395	420	397	370	151	17-1	330	422	465	505	173*
7-2	360	325	—	256	139	17-2	345	420	378	408	171*
7-3	355	440	—	285	125	17-3	367	460	458	502	174*
7-4	370	442	342	330	169*	17-4	370	475	430	400	155
7-5	290	382	447	325	178*	17-5	365	455	508	545	176*
7-6	395	420	318	255	155	17-6	345	467	535	580	171*
8-1	345	375	—	312	89	18-1	370	530	657	685	163*
8-2	330	432	322	272	167	18-2	357	445	468	462	164*
8-3	350	360	408	330	172	18-3	365	370	302	268	154*
8-4	365	380	—	265	113	18-4	317	482	558	588	165*
8-5	345	400	392	370	170*	18-5	337	550	618	520	165*
Experimental mean.....	331	386	364	301	142	t.....	0	4.7	4.41	7.08	—
						n.....	32	32	23	32	—
Control mean.	331	455	512	511	161						
						P.....	1	<.01	<.01	<.01	—
Difference....	0	69	148	210	19						

* Killed.

Group 6: Production of intestinal tuberculosis by oral infection: Control and chronic vitamin-C deficient animals were fed 5 times weekly with 0.25 cc. of the infecting material by means of a tuberculin syringe. The infecting material was either a heavy suspension of tubercle bacilli, H37, grown for 2 weeks on synthetic media (Proskauer and Beck), or sputum with a very high Gaffky count.

A: Animals fed tubercle bacilli, H37: Two groups of 17 guinea pigs, each having a mean weight of 331 gm., were placed on the partially depleted vitamin-C and control diets. At the same time feeding was begun with culture H37.

Pathology: The survival period, though apparently not significantly different according to the means seen in table 15, really was significant. At 159 days, 7 partially depleted and 14 control animals were living.

TABLE 16

Scurvy findings in animals fed tubercle bacilli, H37

ANIMAL NUMBER	CLINICAL SYMPTOMS	BONY SYSTEM			HAEMORRHAGES				SCORE
		Teeth	Ribs	Joints	Ribs	Intestine	Joints	Muscle	
Experimental									
6-1	mild	2	1	½	1	1	0	0	5½
6-2	mod.	4	3	1	1	0	0	2	11
6-3	0	0	0	0	0	0	0	0	0
6-4	v. mild	0	0	2	0	0	0	0	2
6-5	mild	3	½	0	0	0	0	0	3½
6-6	0	0	0	0	0	0	0	0	0
7-1	mod.	3	3	0	1	0	0	1	8
7-2	0	1	0	0	0	0	0	0	1
7-3	mod.	4	3	1	0	1	0	0	9
7-4	severe	3	4	2	1	4	0	3	17
7-5	mild	2	1	0	0	0	0	0	3
7-6	0	2	1	0	0	0	0	0	3
8-1	mild	4	1	1	0	0	0	0	6
8-2	severe	2	1	0	1	0	0	1	5
8-3	severe	3	3	1	0	0	0	3	10
8-4	mod.	2	1	0	0	0	1	0	4
8-5	mod.	3	3	0	0	0	0	4	10
Control	0	0	0	0	0	0	0	0	0

The progressive killing-off of animals was begun and the last animal killed at 178 days. In this period, 3 partially depleted vitamin-C animals died naturally, but the controls, all in good physical condition, lived for the duration of the experimental period. Table 16 presents the scurvy score.

Generalized tuberculous involvement was present to a greater extent in the chronic scorbutic than in the control group (table 17). Twelve of 17 deficient animals (70 per cent) had tuberculosis scores of 3 plus or more, while only 8 of 17 (47 per cent) of the controls had a similar score.

TABLE 17

Macroscopic pathology on animals fed H37

ANIMAL NUMBER	LUNG	LIVER	SPLEEN	LYMPH NODES						SMALL INTESTINE	CAECUM	LARGE INTESTINE	TB. SCORE
				Ing	R-p	T-b	Hep	Mes	Cer				
Experimental													
6-1	1+	1+?	0	0	0	0	0	2*	2*	0	tb	0	1+
6-2	3+	2+	3+	1	0	0	0	4*	0	0	0	0	3+
6-3	3+	4+	4+	0	0	4**	3**	4**	4*	0	0	0	4+
6-4	4+	1+	1+	1	1	1	2	3**	1	0	0	tb	3+
6-5	4+	2+	4+	1**	1	2**	3*	4**	4*	tb	0	0	4+
6-6	1+	2+	4+	1*	0	0	2	3*	3*	tb	ulcer	0	3+
7-1	1+	1+	1+	0	0	1*	1	8**	3*	tb	0	0	2+
7-2	3+	2+	2+	2*	0	—	1*	4*	4*	0	0	0	4+
7-3	4+	2+	4+	1*	2**	1*	3*	4**	3*	0	0	ulcer	4+
7-4	1+	1+	3+	1**	0	0	0	2*	2*	0	0	0	2+
7-5	2+	1+	4+	1	1	1*	1*	4**	3**	tb	0	0	3+
7-6	3+	1+	1+	1**	2**	2*	1**	2*	0	0	0	0	3+
8-1	1+	1+	1+	1**	1**	1	1	4**	3**	0	0	tb	2+
8-2	3+	2+	3+	0	0	1**	0	1	1	0	0	0	4+
8-3	1+	1+	1+	0	0	0	0	2	1	tb	0	0	1+
8-4	2+	2+	2+	0	0	3	3	4	3	0	0	0	3+
8-5	4+	1+	3+	0	0	1	0	2	3*	tb	0	0	3+
Control													
16-1	4+	1+	2+	0	2*	4**	3**	5**	1	0	0	0	3+
16-2	4+	2+	4+	0	0	4*	2*	9**	3*	0	0	0	4+
16-3	2+	1+	2+	1	1	2	3	4	2	0	0	0	2+
16-4	1+	0	0	0	0	0	1	3	0	0	0	0	1+
16-5	2+	0	2+	1	0	0	3	3	3*	0	0	tb	2+
16-6	1+	0	1+	0	0	1	0	3**	4**	0	0	0	1+
17-1	4+	1+	2+	1	1	4	2	6	2	0	tb	tb	3+
17-2	4+	3+	1+	0	0	3**	0	2**	2*	0	0	0	3+
17-3	4+	1+	1+	0	0	2**	0	2**	1**	0	0	0	3+
17-4	4+	1+	1+?	0	0	2*	1*	2**	2**	0	0	0	3+
17-5	1+	0	0	0	0	3**	1**	3**	2**	0	0	0	1+
17-6	1+	0	1+	0	0	4	2**	8	2	0	0	0	2+
18-1	1+	0	1+	1	0	4**	2	4**	4**	tb	0	0	2+
18-2	2+	1+	3+	1	1	3	3	7**	3	tb	0	0	3+
18-3	3+	1+	1+	0	0	2**	2**	2**	0	tb	0	0	2+
18-4	2+	1+?	1+	0	0	4	0	2	2**	0	0	0	2+
18-5	3+	1+	1+	0	0	1	1	1	1	0	0	tb	3+

* Caseation.

** Calcification.

The involvement of the lungs was practically the same in both groups. However, in the liver and spleen there was greater involvement in those receiving an insufficient quantity of the vitamin. The mesenteric lymph

TABLE 18

Microscopic pathology of the intestinal tract of animals fed strain H37

ANIMAL NUMBER	TB. SCORE	SMALL INTESTINE	CAECUM	LARGE INTESTINE	SCURVY SCORE	SURVIVAL
Experimental						
6-1	1	—†	1+	0	5½	138
6-2	3	tb 2+	0	0	11	178
6-3	4	0	0	0	0	85
6-4	3	—	0	1+*	2	108
6-5	4	ulcer*	tb 3+*	—	3½	161
6-6	3	ulcer*	ulcer	0	0	125
7-1	2	tb 3+	0	0	8	151
7-2	4	—	0	0	1	139
7-3	4	—	0	ulcer*	9	125
7-4	2	tb 3+	0	0	17	169
7-5	3	tb 3+	0	0	3	178
7-6	3	0	0	0	3	155
8-1	2	0	0	tb 2+	6	89
8-2	4	ulcer*	0	0	5	167
8-3	1	tb 3+	0	0	10	172
8-4	3	—	0	0	4	113
8-5	3	ulcer*	0	0	10	170
Control						
16-1	3	0	0	0	0	161
16-2	4	tb 2+	0	0	0	162
16-3	2	tb 2+*	0	0	0	159
16-4	1	0	—	0	0	159
16-5	2	—	0	tb 2+	0	113
16-6	1	0	0	0	0	161
17-1	4	0	tb 1+	tb 2+	0	173
17-2	4	0	0	0	0	171
17-3	3	0	0	0	0	174
17-4	3	0	0	0	0	155
17-5	1	0	0	0	0	176
17-6	2	0	0	0	0	171
18-1	2	tb 1+*	0	0	0	163
18-2	3	tb 3+*	0	0	0	164
18-3	2	tb 2+*	0	0	0	154
18-4	2	0	0	0	0	165
18-5	3	tb 1+*	0	tb 1+*	0	165

* Tubercle bacilli found.

† — indicates sections unsuitable for examination.

nodes were 8 to 9 times larger than normal, and were caseous. This condition was common to both groups. Organisms were found to the same extent in similar organs of comparable groups.

Eleven gross lesions were found in the intestinal tract of 8 of the partially depleted animals, and 7 lesions in 6 of the controls. With the exception of two 3-mm. ulcerations in the chronic scorbutic animals, the type of lesion was similar in both groups, that is, small pin-head, white areas in the Peyer's patches and lymph follicles.

Microscopically, the intestinal lesions of the chronic scorbutic animals were more extensive and more necrotic than those of the controls (table 18). The chronic vitamin-C deficient guinea pigs showed lesions in 13 of 17 animals (76.5 per cent) as contrasted to 8 of 17 animals (41 per cent) in the control group. Ulceration was found in 6 of the deficient group but in none of the controls. In both series, lesions were more frequently found in the ileum. The small intestines of 9 animals in the scorbutic group showed lesions. The sections from 3 animals were negative and those of 5 others were unsuitable for examination. Thus, of 12 chronic scorbutic animals examined histologically, 9 had lesions. Only 6 of the control animals examined had lesions of the small intestine. In the caecum, 3 partially depleted animals showed lesions; one an ulcer, one a 3-plus lesion, and the third a minimal 1-plus lesion. In the control group 1 minimal 1-plus lesion was noted. In the colon 3 of 13 animals on the diet low in vitamin C had lesions and 1 of these was an ulcer. Among the controls 3 of 17 showed lesions.

The weight difference (table 15 and figure 5) between the comparable groups was of definite significance. Tuberculin tests, 25 and 51 days after the feeding of the culture was begun, showed little variation.

Guinea pigs fed culture H37 and maintained on a diet partially depleted in vitamin C showed significant differences from similar animals on the ample diet. Their weights and survival periods were less. Greater involvement with generalized and intestinal tuberculosis occurred. They also showed ulceration of the intestinal mucosa which did not occur in the control group.

B: Sensitized animals fed strain H37: These guinea pigs were sensitized by subcutaneous inoculation with 0.1 mgm. of the avirulent strain R1 and at the same time were divided into two groups, 11 animals being placed on the chronic scurvy producing diet and 12 on the control diet. Feeding with culture H37 was begun 3 weeks later and this was done similarly to group 6a.

Pathology: There was practically no difference in the macroscopic pathology of tuberculosis (table 19). However, it should be noted that

the survival period as well as the weights of the chronic scorbutic animals were significantly less than those of the controls (table 20). Minimal or moderate involvement occurred in both groups, although the

TABLE 19

Autopsy findings on animals sensitized with strain R1 and fed strain H37

NUMBER OF ANIMAL	LUNG	LIVER	SPLEEN	LYMPH NODES						INTESTINE‡	TB. SCORE	SCURVY SCORE	SURVIVAL
				Ing	R-p	T-b	Hep	Mes	Cer				
Experimental													
42-1	0	0	0	1*	0	0	0	0	0	0	1	5	143
42-2†	1+	0	1+	2*	0	0	0	2*	0	tb	2	7	200
42-3	1+	1+	1+	1	0	0	0	0	0	0	2	4	53
42-4	tb. pn.	0	0	0	0	0	0	0	0	0	3	½	112
42-5	1+	1+	1+	1**	0	0	0	1	0	0	2	9	48
42-6	0	0	0	1**	0	0	0	0	0	0	1	9	107
43-1	0	1+	1+	1*	0	0	0	0	0	0	1	3	87
43-2	0	1+	1+	0	0	0	0	0	0	0	1	7½	112
43-3	3+	1+	1+	1*	0	0	0	0	0	0	3	6	109
43-4	1+	P.E.	0	1*	0	0	0	0	0	0	1	4	56
43-5	1+	1+	1+	3*	0	0	0	1	1	0	2	6	43
Control													
40-1	pl	0	0	1*	0	0	0	0	0	0	1	0	179
40-2†	3+	2+	1+	1*	0	4	1	2	2	0	3	0	204
40-3†	1+	0	1+	1	0	2	0	3*	1	tb	1	0	204
40-4	0	1+	1+	3*	2*	0	0	1	3*	tb	1	0	95
40-5	1+	1+	1+	1	0	3*	0	1	0	0	2	0	119
40-6†	1+	0	1+	0	0	2*	0	0	0	0	1	0	204
41-1	3+	0	2+	1*	0	1	0	1	0	0	3	0	107
41-2†	1+	1+	1+	1	0	3	1	3	0	tb	2	0	200
41-3	1+	1+	1+	2*	0	0	0	1	0	0	2	0	60
41-4	1+	0	1+	1**	0	0	0	0	0	0	1	0	163
41-5†	2+	0	1+	1	0	4	0	3	0	tb	2	0	200
41-6	1+	0	1+	1	0	0	0	1*	0	0	1	0	141

* Caseation.

** Calcification.

† Killed.

‡ Lesions were found in the small intestine.

Cons. = consolidation; P.E. = plastic exudate; pl. = pleurisy; pn. = pneumonia.

controls showed more extensive lesions in the mesenteric lymph nodes. Gross lesions were found only in the small intestine, 4 among the controls and 1 among the deficient animals.

Microscopically, 6 control guinea pigs had lesions of the small intestine

as compared to 3 of the chronically deficient group (table 21). No advanced lesions or ulcers were found in the controls, but 1 chronic

TABLE 20

The weights and survival period of guinea pigs sensitized with strain R1 and fed strain H37

ANIMAL NUMBER	ONSET	FED H37	ON H37: WEIGHTS IN GRAMS AT				SURVIVAL IN DAYS
			4 weeks	8 weeks	16 weeks	Death	
Experimental							
42-1	332	325	320	355	270	258	143
42-2*	308	290	260	358	385	312	200
42-3	375	375	348	—	—	248	53
42-4	347	355	348	415	—	360	112
42-5	312	305	—	—	—	210	48
42-6	314	315	265	300	—	237	107
43-1	352	380	300	—	—	258	87
43-2	353	367	325	380	—	295	112
43-3	387	365	280	275	—	257	109
43-4	315	335	270	—	—	270	56
43-5	265	265	—	—	—	208	42
Control							
40-1	257	315	355	365	330	212	179
40-2*	283	335	352	382	528	530	204
40-3*	230	302	353	388	556	540	204
40-4	330	320	345	318	—	282	95
40-5	340	402	480	540	—	530	119
40-6*	398	390	415	420	528	538	204
41-1	365	418	488	457	—	333	107
41-2*	375	400	463	445	518	560	200
41-3	336	340	330	—	—	315	60
41-4	298	338	395	365	282	260	163
44-5*	370	412	482	440	470	535	200
44-6	330	365	420	390	358	305	141
Experimental mean....	331	334	291	347	327	228	97
Control mean.....	326	361	406	410	459	412	156
Difference.....	5.2	27	115	63	132	184	59
t.....	0.275	1.63	4.79	9	1.59	4.1	2.87
n.....	21	21	19	15	8	21	21
P.....	0.78	0.12	<0.01	<0.01	0.16	<0.01	<0.01

* Killed.

vitamin-C animal had a 3-plus lesion. One minimal lesion was noted in the caecum of a control and none among the chronic scorbutic group. There was a 1-plus lesion in the colon of one of the latter animals.

It will be noted that 8 control and only 2 deficient animals were living at 16 weeks. And also, a survival period of 200 days seemed necessary for the development of intestinal lesions in the majority of these sensitized animals. This apparently accounts for the slightly higher incidence of lesions in the control group. The tuberculin tests were not significantly different.

TABLE 21

Microscopic pathology of the intestinal tract of animals sensitized with R1 and fed H37

ANIMAL NUMBER	SURVIVAL	TB. SCORE	SMALL INTESTINE	CAECUM	LARGE INTESTINE
Experimental					
42-1	143	1+	0	0	0
42-2	200	2+	tb 2+; 3+*	0	0
42-3	53	1+	0	0	0
42-4	112	3+	0	0	0
42-5	48	1+	0	0	0
42-6	107	1+	0	0	0
43-1	87	1+	0	0	0
43-2	112	1+	tb 1+	0	0
43-3	109	3+	0	0	0
43-4	56	1	0	0	0
43-5	43	2+	0	0	tb 1+
Control					
40-1	179	1+	—	—	—
40-2	204	3+	tb 1+	0	0
40-3	204	1+	tb 1+; 2+	0	0
40-4	95	1+	tb 1+*	tb 1+*	0
40-5	119	1+	0	0	0
40-6	204	1+	tb 2+; 2+	0	0
41-1	107	3+	0	0	0
41-2	200	2+	tb 1+*	0	0
41-3	60	1+	0	0	0
41-4	163	1+	0	0	0
41-5	200	2+	tb 1+*	0	0
41-6	141	1+	0	0	0

* Tubercle bacilli found.

The guinea pigs on an inadequate amount of vitamin C had tuberculosis similar to that in nonscorbutic animals who died at a significantly later date. The exception to this was a slightly greater number of intestinal lesions in the control group which apparently lived sufficiently long for such lesions to develop. Sensitization with R1 seemed to afford

the same degree of protection against generalized tuberculosis to both groups.

C: Sputum-fed animals: Guinea pigs were divided into a chronic vitamin-C deficient group of 14 and a control group of 17 animals. At the same time, feeding with sputum was begun.

Pathology: The survival periods and weights are given in table 22. It will be noted that the differences in the means of the weights were significant from 12 weeks until death. Within 168 days of feeding, 10 of 17 control animals had died but 4 had been killed for pathological study. Only 1 of 14 deficient animals was alive. The experiment was terminated at 196 days.

In the chronic scorbutic group 14 animals lived 6 weeks or more. Two of these died in the 8th week, one of peritonitis and the other of pneumonia. Only 1-plus tuberculosis was present macroscopically. Considering a 3- or 4-plus score as generalized tuberculosis, 9 of 14 chronic scorbutic animals (65 per cent) had diffuse generalized involvement as compared to 6 of 17 control animals (35 per cent). The mesenteric lymph nodes of the chronic vitamin-C depleted animals showed caseation in all but 2 animals. The findings were practically the same in the control group (table 23).

The intestinal tract of 11 of 14 animals in the chronic scorbutic group showed gross lesions as contrasted to 11 of 17 controls. However, in the scorbutic group, ulcers were present in six. The control group showed ulceration in only one animal.

Microscopically (table 24), the lesions varied from a localized mononuclear tubercle, to diffuse infiltration, necrosis and ulceration (figures 8–9). The deficient group showed much more extensive and severe involvement of the small intestine than the group of nonscorbutics. Excluding animals 64–2 and 64–3, which lived only 68 and 63 days respectively, 8 of 12 chronically scorbutic animals (66 per cent) had ulcers in the small intestine, as contrasted to 4 of 16 (25 per cent) controls. In the caecum, lesions were present in 4 of 12 partially depleted and in 1 control animals. One animal with chronic scurvy had an ulcer and 2 had less advanced lesions while only 1 control animal had a lesion in the colon.

Fourteen animals on the diet producing chronic scurvy gave a tuberculin score of 30 and 17 controls a score of 48, at 30 days. Tests at 112 days were similar in both groups.

TABLE 22

The weights and survival of sputum-fed animals

NUMBER OF ANIMAL	WEIGHT IN GRAMS AT						SURVIVAL IN DAYS
	Onset	6 weeks	12 weeks	18 weeks	24 weeks	Death	
Experimental							
63-1	465	600	555	410	—	405	163
64-2	418	445	—	—	—	490	68
64-3	426	455	—	—	—	405	63
64-4*	345	465	365	425	355	370	175
64-5	335	480	430	—	—	345	101
65-1	442	455	430	455	—	445	140
65-2	365	376	315	—	—	260	94
65-3	550	500	480	435	—	370	145
65-4	448	468	390	—	—	345	96
65-5	455	490	415	—	—	365	103
65-6	430	480	550	430	—	385	139
66-2	540	620	555	445	—	360	144
72-1	510	622	495	—	—	375	94
72-2	485	570	570	518	—	455	145
Control							
67-1*	475	535	625	590	470	465	175
67-2	485	530	580	370	315	315	168
67-3*	485	400	530	580	485	515	196
67-4	375	415	510	—	—	560	117
67-5	655	450	—	—	—	445	77
67-6*	305	315	380	—	—	425	98
67-7	435	467	490	485	—	375	153
68-2	518	550	560	—	—	360	109
68-4*	445	470	525	—	—	550	105
68-5*	412	525	495	—	—	545	101
68-6	455	500	476	405	—	405	128
68-7	430	405	420	485	—	340	150
69-1*	518	575	570	—	—	535	98
69-2*	530	505	560	545	605	600	196
70-2	495	635	555	655	695	500	192
71-1*	490	515	545	555	505	425	196
71-2*	660	650	815	680	700	760	196
Experimental mean....	444	502	454	445	355	384	119
Control mean.....	480	495	539	535	539	478	142
Difference.....	36	7	85	90	184	94	23
t.....	1.27	.26	2.45	2.27	1.24	2.91	1.64
n.....	29	29	26	15	6	29	29
P.....	0.22	0.79	0.021	0.027	0.26	<0.01	0.11

* Killed.

TABLE 23

Macroscopic pathology on sputum-fed animals

NUMBER OF ANIMAL	LUNG	LIVER	SPLEEN	LYMPH NODES						SMALL INTESTINE	CAECUM	COLON	TB. SCORE	SCURVY SCORE	SURVIVAL
				Ing	R-p	T-b	Hep	Mes	Cer						
Experimental															
63-1	1+	3+	3+	1	0	0	2*	3*	0	ulcer	0	0	3+	7	163
64-2†	pl. tb 0	0	0	0	1	0	0	1	0	0	0	0	1+	0	68
64-3‡	cons.	0	1+	0	0	0	0	0	0	0	0	0	1+	0	63
64-4	2+	3+	4+	0	0	2*	2	4*	2	tb	tb	0	4+	1	175
64-5	1+	2+	0	0	1	0	3*	4*	0	ulcer	0	0	2+	4	101
65-1	3+	4+	4+	0	0	1	0	3*	0	ulcer	0	0	4+	½	140
65-2	0	1+	3+	1	0	2*	3*	2*	0	tb	0	0	3+	3	94
65-3	1+	2+	2+	0	0	0	1	3	1	tb	tb	0	3+	4	145
65-4	1+	0	0	0	0	0	2*	0	0	0	0	0	1+	1	96
65-5	1+	2+	3+	0	0	1	3*	4*	0	ulcer	0	0	3+	2	103
65-6	3+	4+	4+	2*	1	1*	2*	3*	3*	ulcer	tb	ulcer	4+	5	139
66-2	2+	2+	4+	2*	1	1*	1	3*	2	ulcer	0	0	4+	½	144
72-1	0	0	0	0	0	0	0	0	0	tb	0	0	1+	2	94
72-2	1+	2+	4+	0	0	0	1*	2*	0	tb	0	0	4+	5	145
Control															
67-1	2+	1+	3+	0	0	2	2*	2*	0	tb	tb	0	3+	0	175
67-2	3+	2+	3+	0	1	3*	3*	4*	1*	tb	0	0	4+	0	168
67-3	2+	2+	3+	0	0	3	2*	3*	2	tb	0	0	3+	0	196
67-4‡	cons.	0	0	0	0	0	2*	3*	0	0	0	0	1+	0	117
67-5	pl. tb 0	0	1+	0	0	0	0	2*	0	0	0	0	1+	0	77
67-6	2+	0	1+	0	0	3*	2*	4*	0	tb	0	0	2+	0	98
67-7	1+	1+	1+	0	0	0	2	4*	0	tb	0	0	2+	0	153
68-2	0	0	0	0	0	0	2*	2*	4*	tb	0	0	1+	0	109
68-4	1+	0	2+	0	0	0	2*	4*	0	tb	tb	0	2+	0	105
68-5	2+	0	2+	0	0	2	3*	4*	0	0	0	0	2+	0	101
68-6	1+	1+	1+	0	0	2*	3*	4*	1	0	0	0	1+	0	128
68-7	1+	1+	1+	0	0	3	3*	3*	0	tb	0	0	2+	0	150
69-1	0	1+	1+	0	0	0	3*	4*	4*	0	0	0	1+	0	98
69-2	2+	2+	1+	0	1	3*	2	4*	0	tb	tb	0	3+	0	196
70-2	3+	1+	2+	0	0	3	0	3*	2*	tb	tb	0	3+	0	192
71-1	3+	1+	2+	0	0	2	0	3*	2*	ulcer	0	0	3+	0	196
71-2	2+	0	1+	0	0	3*	1	2	0	0	0	0	2+	0	196

* Caseation.

† Died of peritonitis.

‡ Died of pneumonia.

Cons. = consolidated; pl. = pleurisy.

TABLE 24

Microscopic pathology of the intestines of sputum-fed animals

NUMBER OF ANIMAL	TB. SCORE	SMALL INTESTINE	CAECUM	LARGE INTESTINE	SURVIVAL
Experimental					
63-1	3	ulcer*	0	0	163
64-2	1	0	0	0	68
64-3	1	0	0	0	63
64-4	4	3+*	1+*	1+	175
64-5	2	ulcer*	0	0	101
65-1	4	ulcer*	0	0	140
65-2	3	3+*	0	0	94
65-3	3	ulcer* ulcer*	2+*	1+	145
65-4	1	0	0	0	96
65-5	3	ulcer* 3+*	0	0	103
65-6	4	ulcer*	3+*	ulcer*	139
66-2	4	ulcer*	0	0	144
72-1	1	3+*	1+	0	94
72-2	4	ulcer* 3+*	0	0	145
Control					
67-1	3	ulcer*	3+*	0	175
67-2	4	ulcer* 3+*	0	0	168
67-3	3	1+*	0	0	196
67-4	1	2+	0	0	117
67-5	2	3+	0	1+	77
67-6	2	0	0	0	98
67-7	2	1+*	0	0	153
68-2	1	1+*	0	0	109
68-4	2	2+*	0	0	105
68-5	2	0	0	0	101
68-6	1	2+*	0	0	128
68-7	2	—	—	—	150
69-1	1	1+	0	0	98
69-2	3	ulcer*	0	0	196
70-2	3	1+*	0	0	192
71-1	3	ulcer*	0	0	196
71-2	2	1+	0	0	196

* Tubercle bacilli found.

Involvement of the intestine was localized to a small area, usually a Peyer's patch or lymph follicle. Sections were made from grossly involved areas in preference to negative areas at random.

1-plus = localized infiltration of epithelioid cells—early tubercle; 2-plus = infiltration with beginning necrosis; 3-plus = epithelioid cells, lymphocytes, fibroblasts, with necrosis; 4-plus = ulceration of the mucosa in addition to the 3-plus.

PLATE 1

Fig. 1. Mean average weights of uninoculated diet control animals. The continuous line indicates the control animals; the broken line the chronic scorbutic animals.

Fig. 2. Mean average weights of animals inoculated intraperitoneally with strain H37. The continuous line indicates the control animals; the broken line the chronic scorbutic animals.

Fig. 3. Mean average weights of animals inoculated subcutaneously with 0.1 mgm. of strain H37. The continuous line indicates the control animals; the broken line the chronic scorbutic animals.

Fig. 4. Mean average weights of animals inoculated subcutaneously with strain R1. The continuous line indicates the control animals; the broken line the chronic scorbutic animals.

Fig. 5. Mean average weights of animals fed strain H37. The continuous line indicates the control animals; the broken line the chronic scorbutic animals.

Fig. 6. Mean average weights of animals sensitized with strain R1 and 3 weeks later fed strain H37. The continuous line indicates the control animals; the broken line the chronic scorbutic animals.

Fig. 7. Mean average weights of animals fed infected sputum. The continuous line indicates the control animals; the broken line the chronic scorbutic animals.

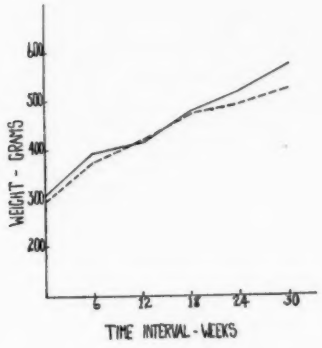

FIG. 1

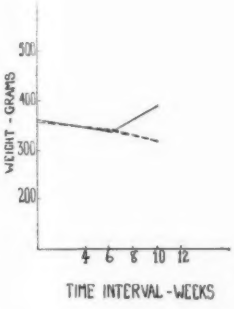

FIG. 2

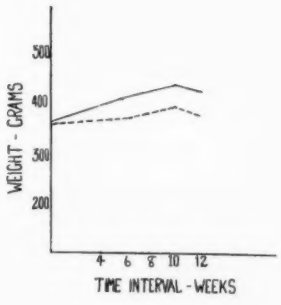

FIG. 3

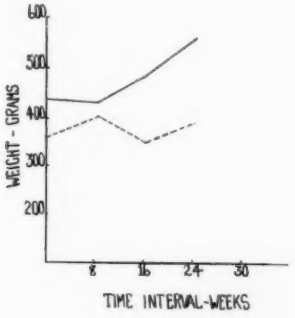

FIG. 4

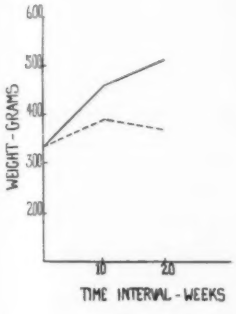

FIG. 5

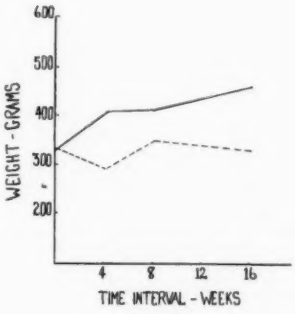

FIG. 6

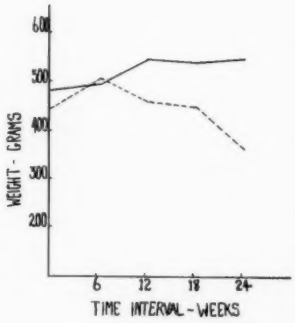

FIG. 7

PLATE 1

FIG. 8

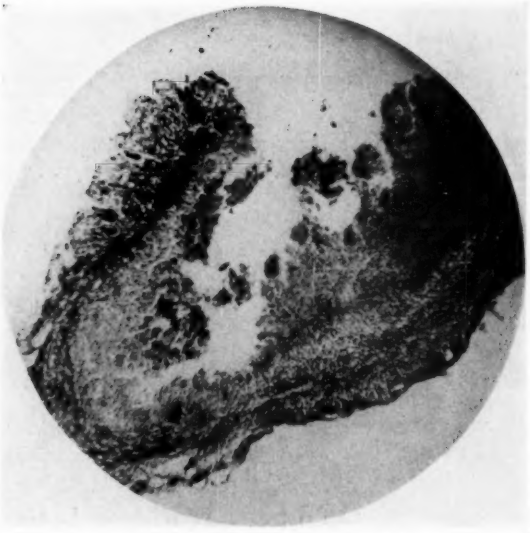

FIG. 9

PLATE 2

Fig. 8. Ulceration of the mucosa in the small intestine. 60×
Fig. 9. Ulceration of the mucosa with penetration into the submucosa. 60×

A considerably higher percentage of the animals maintained in a chronic state of scurvy and fed sputum, developed generalized tuberculosis and ulcers of the intestinal tract than did control animals. The chronic scorbutic guinea pigs also had a shorter survival period and a great decrease in body weight.

DISCUSSION

Guinea pigs placed on a diet completely deficient in vitamin C die within 4–5 weeks. However, when this diet is supplemented with small quantities of an antiscorbutic substance, animals can be maintained in a state of chronic scurvy for several months. Our animals were kept on this diet during an experimental period of 7 months. One of these animals developed a positive tuberculin test and at death (197 days) early tuberculous lesions were found. Lurie (1930) showed that normal guinea pigs kept in the same room, but not in the same cage with infected animals, may develop tuberculosis in 9 per cent of the cases after 6 months. Since most of our experiments were terminated about the 6th month, the possibility of exogenous infection in our experimental groups was practically eliminated.

The inoculating dose used for infecting animals by the intraperitoneal route was apparently sufficiently large to mask any effect of the chronic vitamin-C diet, since the survival period, weights, and pathological changes did not differ significantly from those of the controls.

The combination of a partial depletion of vitamin C and progressive tuberculous infection apparently causes a significant shortening of the survival period of guinea pigs. This is shown in the animals inoculated subcutaneously and by feeding. Thus, the experiments of Mouriquand and co-workers (1925), Bieling (1924), and Heymann (1926) and Basu (1934) were confirmed.

When young animals (250 gm. in group 3B) were inoculated, or when the infecting dose of tubercle bacilli was large (intraperitoneal group), weight differences between the chronic scorbutic and nonscorbutic animals did not occur. However, significant weight differences were present between tuberculous animals on a partially depleted vitamin-C ration and control animals, when the animals were older and their tissues less rapidly invaded by virulent bacilli. In the very young animals, however, the smaller quantity of organisms used did not compensate for the age factor.

Bieling (1924) and Schilf (1923) report decreased skin sensitivity to tuberculin in tuberculous animals on an acute scurvy diet. Our findings were inconsistent. The partial lack of the vitamin in the diet appeared

to result in a weaker reaction to tuberculin in animals infected by feeding sputum or in those subcutaneously inoculated with R1 or with 0.012 mg. of H37, but not in the other groups. These latter were the groups inoculated subcutaneously with 0.1 mgm. of strain H37 and those fed the same organism.

As we were interested in the survival period, a chronic deficient or a control animal was usually not killed when a death occurred in the contrasting group. Exception to this occurred when the deficient group died within a short space of time (as did the acute scurvy) and the control series was then killed, or when surviving animals in both groups were killed at the termination of the experiment. However, with the exceptions mentioned earlier, the chronically deficient animals died at an earlier date with a similar type of infection and the same as or a greater extent of tuberculous involvement than was found at a significantly later period in the nonscorbutic animals. It was therefore considered reasonable to assume that the progress of tuberculosis was more rapid in the partially depleted animals. This was especially evident in the case of sputum-fed animals, in which generalized and intestinal tuberculosis was much more extensive in the group not receiving an ample supply of the vitamin. Baldwin and Gardner (1921) demonstrated generalized tuberculosis in 5 of 40 animals given a normal diet and R1 infection by inhalation. They attributed this to the poor condition of the animals, some of which were half-grown. We obtained no apparent increase of virulence in this strain of the tubercle bacillus even though our animals were on a diet producing chronic scurvy.

Intestinal lesions were found in many of our animals infected by the enteric route. This was anticipated from the findings of Redman (1922), Griffith (1924), Medlar and Sasano (1924–25), Lurie (1930) and McConkey and Smith (1933). A greater number of lesions were revealed by microscopic than macroscopic examination. The greater number of sections taken the more frequently were lesions found in intestines that were grossly negative. Lesions were usually found in the lymphoid tissue. When ulcers were present, they were usually in the ileum.

In our experiments, ulceration of the mucosa occurred much more frequently when infected tuberculous sputum was fed than when a pure culture of tubercle bacilli was fed. The presence of other organisms in sputum may be of significance as well as a difference in the virulence of the tubercle bacilli. Tuberculous lesions and ulcers developed much oftener in the animals on the diet producing a state of chronic scurvy. McConkey and Smith (1933) found a considerably higher proportion of open ulcers in such animals than we did. Several factors in their

experiments differed from ours. The amount of sputum given was 0.5 cc. while we gave 0.25 cc. The constituents of the diet were not the same. These workers suggest their diet to be relatively deficient in vitamin A in addition to a deficiency in the C factor. However, as hay was given *ad lib.* and a good quality contains a large amount of vitamin A (Russell, 1929; Smith and Briggs, 1933; Meigs and Converse, 1933), that deficiency was probably not present. All animals in one of their groups were sensitized with R1 before sputum was fed. The second series, in which an even greater number of ulcers developed, had been fed 3 times with virulent H37 a month before sputum feeding began. The only exception to this was 10 animals on a diet containing cabbage who showed tuberculous lesions but no ulcers. Therefore, with the exception noted, all animals were probably sensitized. The surviving animals in our sputum fed group were not killed until 6½ months.

The same degree of protection was afforded partially depleted and control animals sensitized with R1 strain and then fed the pure culture of H37, as far as the development of generalized and intestinal tuberculosis was concerned. However, since most of the chronic scorbutic animals did not live long enough for the development of lesions in the intestinal tract, these findings cannot be compared in the 2 groups. We have been unable to explain why this series of guinea pigs died earlier than the nonsensitized group which was also fed H37, inasmuch as less tuberculosis was present in the sensitized group.

CONCLUSIONS

1. Chronic vitamin-C deficiency combined with a progressive tuberculous infection causes a significant shortening of the survival period and a significant decrease in the body weight of guinea pigs.

2. Chronic scurvy did not cause the development of generalized tuberculosis in animals infected with the relatively avirulent strain, R1.

3. Generalized tuberculosis develops more rapidly in chronic vitamin-C deficient animals than in nonscorbutic animals, infected by the subcutaneous or enteric route with strain H37 or tuberculous sputum.

4. Guinea pigs maintained on a partially depleted vitamin-C diet develop more lesions and ulcers in the intestinal tract than do animals on a complete diet when fed tubercle bacilli.

5. When infected tuberculous sputum is fed, the extent and severity of the tuberculosis in the intestinal tract is greater than when pure cultures of tubercle bacilli are administered.

6. Animals infected with tuberculosis and allowed to develop acute scurvy show no more tuberculosis than the control guinea pigs.

We wish to thank Dr. Alexander Wiener of the Pathology Department for his valuable assistance in the statistical analysis of our data.

We are especially grateful to Mead Johnson and Company whose generous assistance made this work possible.

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THE EFFECTS OF INTRAVENOUS INJECTIONS OF LECITHIN ON TUBERCULOSIS IN THE RABBIT[1, 2]

EDNA H. TOMPKINS

Tuberculosis may be regarded as a disease which primarily involves the phagocytic mononuclear cells of the blood and tissues and so modifies those cells that they develop into epithelioid and giant cells. Cunningham, Sabin, Sugiyama and Kindwall (6) demonstrated the development of epithelioid and giant cells from monocytes in tuberculosis of the rabbit and found that the circulating monocytes are increased. Camp, Luton, Tompkins and Cunningham (3) obtained similar findings in the blood of tuberculous guinea pigs, and numerous reports of supravital studies of the blood of human cases of tuberculosis, the earliest of which was made by Morriss and Tan (14), indicate an identical involvement of the monocytes in man. Tompkins and Cunningham (21) demonstrated that epithelioid cells develop from clasmatocytes, as well as from monocytes, in tuberculosis.

While dominantly characteristic of tuberculosis, the conversion of the phagocytic mononuclear cells into epithelioid and giant cells is not specific to that disease and has been found to occur after the introduction of numerous foreign substances into the connective tissues. Tompkins (22) made supravital studies of the reactions of the subcutaneous tissues to lipides and various other foreign substances, and reviewed the literature concerning similar studies. She found that the reactions following the injection of many foreign bodies and the inoculation of tubercle bacilli are similar to one another, and differ from the reactions to the lipides. They are characterized by an influx of polymorphonuclear cells followed by the appearance of monocytes and clasmatocytes, which eventually undergo degenerative changes leading to the formation of epithelioid and giant cells. After injections of lecithin prepared from egg-yolk, on the other hand, and particularly of lipides prepared from

[1] From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee. This study was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

[2] The technical assistance of B. Howard Blue, Department of Anatomy, Vanderbilt University School of Medicine, has been of very great help in this work.

spinal cord, there is a very different effect upon the macrophages. They quickly develop to great size and phagocytic activity without changes leading to the formation of epithelioid and giant cells. It was found, further, that when lecithin is mixed with a substance which gives rise to epithelioid cells, it lessens the intensity of that reaction to some extent. Apparently the effect of lecithin upon the phagocytic mononuclear cells is the morphological, and probably physiological, opposite of the effect produced upon the same cells by injections of many other foreign materials, and by tuberculosis. For this reason it seemed possible that lecithin might be of therapeutic value in the treatment of tuberculosis.

METHODS

Repeated treatment seemed indicated, since the lipides are rather quickly absorbed. Intravenous injections were chosen for that reason, in addition to the fact that they seemed to offer the best method for a generalized systemic dissemination of the emulsions. We have subsequently come to believe that, while a generalized dissemination probably occurred in animals without any marked pulmonary involvement, it failed to occur in animals with extensive involvement. This was probably due to an almost complete removal of the emulsions in the lungs. Rabbits were chosen for these experiments because of the ease with which they may be given repeated intravenous injections. The necessity for this choice is to be regretted because of the high degree of natural resistance of that species to tuberculosis.

At the time that these experiments were started, the highly potent preparations of lipides prepared from brain (22) had not been tested. Consequently these experiments were conducted entirely with the preparation of lecithin made from egg-yolk which was employed by Tompkins (22) in the study of the reactions of the connective tissues to subcutaneous injections. The stock supply was purchased in amounts sufficient to last from one to two months. It was kept in a dark ice-chest and was opened about once a week. Ten per cent emulsions in distilled water were sterilized for 2 hours in an Arnold sterilizer at 100°C. and were kept firmly stoppered in an ice-chest until needed. A fresh supply of emulsion was prepared once a week. A fresh flask was opened for each day's injections.

The experiments were divided into two series. In the first series the experimental animals received lecithin preceding, as well as following, the inoculation, and were killed within two months of inoculation. Su-

pravital studies of the blood were made throughout the entire experimental period. This series of experiments was planned to show the effects of intravenous injections of lecithin upon the white blood cells, and upon the course of experimental tuberculosis in rabbits when the injections were given before, as well as after, inoculation.

In the second series of experiments, lecithin was given only following the inoculations, and the animals were permitted to live for study of the length of life. The inoculum contained about two and one-half times as many organisms as that of the first series. This series was planned to show the effects of lecithin upon the longevity of tuberculous rabbits, as well as upon tuberculous lesions when the injections were started only after infection had occurred.

The routine for the injections of the first series of experiments was as follows. One cubic centimetre of the emulsion was given every other day for two injections; then 2 cc. every other day for the same number of injections; then 3 cc. every third day for three injections, followed by 4 cc. every third day for the same number of injections, and finally by 5 cc. twice a week, until one month following the inoculation. From then to the termination of the experiments, 7 to 8 cc. were given twice a week. Amounts as great as 5 cc. seemed well borne by all the animals, but the larger doses caused temporary collapse in a few instances and death in a few others. The animals which died following the injections proved to have advanced infections of the kidneys, and it is therefore probable that death was due to circulatory failure.

In the second series of animals, the injections of lecithin were started 7 days after the inoculation. At that time 3 cc. of the emulsion were given every three days for two injections, then 4 cc. every two days for three injections, then 5 cc. twice a week for three and a half months, followed by 6 cc. twice a week for one and a half months, at which time the injections were stopped on all surviving animals.

The inoculum was made by grinding together numerous cultures of bovine tubercle bacilli, B410, in a mortar with saline solution. These were subcultures from an original culture obtained from Dr. Sabin at the Rockefeller Institute. They were grown on Petroff's egg medium and the cultures when used varied in age from 3 to 6 months. Different groups of subcultures were used for the two experimental series. The emulsions obtained by grinding were filtered through numerous thicknesses of gauze. The filtrates were shaken thoroughly and diluted. One cubic centimetre of the suspension used for inoculation of the animals

of the first series contained approximately 72,000,000 bacilli (Petroff-Hausser counting chamber); and, of the second series, approximately 164,000,000 bacilli. Ten cubic centimetres were given intravenously to each rabbit. There were 24 experimental and control animals, each, in the first series; and 26 experimental and 23 control animals in the second series. All were males, and all were kept in separate cages under the same routine.

The animals of the first series were killed 8 to 9 weeks after inoculation. Those of the second series which survived longer than 7 months after the inoculation were also killed. Complete autopsies were made on all of the animals of both series and blocks of the organs likely to be involved were saved for fixation. A careful estimate of the grade of gross involvement was made at the time of autopsy.

Studies of the white blood cells of all of the animals of series 1 which received lecithin and of 6 of the controls of that series were made twice a week both before and after inoculation. Control counts were made before starting the experimental procedures. The differential counts were made upon 200 cells in supravital preparations stained with neutral red. The blood was always drawn at an interval greater than 12 hours after an injection of lecithin. This series of counts extended over a period of 3 months preceding and 2 months following inoculation.

EXPERIMENTAL FINDINGS

Blood studies: All data concerning the reactions of the different white cells are based upon the absolute numbers. Changes from the averages obtained previous to any experimental procedures are regarded as positive only when they were sustained and as great as 500 cells.

The injections of lecithin had no consistent effect upon the polymorphonuclear cells. The absolute numbers of lymphocytes increased in about half of the animals (54 per cent) within 3 weeks from the start of the injections. Since these elevations did not become greater with larger doses and the remainder of the animals failed to show a sustained elevation of the lymphocytes at any time, it is probable that those increases which did occur were incidental to some factor other than the lecithin.

In the case of the monocytes, on the other hand, there was a definite but gradual increase in the absolute number of cells in a large percentage of the animals, and this was fairly proportional to the dosage and to the interval of time which had elapsed since the beginning of the injections.

The character of the monocytes remained unchanged. A total of 83 per cent of the animals eventually showed increases in the number of circulating monocytes. The increases varied from about double to as much as twenty times the normal values. They were usually gradual until a new level was reached, at which the counts remained fairly stationary until the period of inoculation. The monocytes began to increase in 17 per cent of the animals 3 weeks after the injections were started, and when the amount of each injection was only 2 to 3 cc. Twenty per cent more of the animals showed a beginning increase in monocytes 4 weeks from the start of the injections, when they were receiving 4 cc. at each injection; and 42 per cent, 5 weeks from the start of the injections, when they were receiving 4 to 5 cc. Eight per cent of the animals which failed to show a sustained elevation of the monocytes (17 per cent) did show repeated peaks, leaving only 9 per cent without any increase consequent to the injections of lecithin.

Transitory elevations of the total white counts and the monocytes above the preinoculation levels occurred in most of the experimental animals about 12 days after the inoculation. At the same time the lymphocytes decreased slightly in 50 per cent of the animals. Otherwise there were no consistent changes in any of the white blood cells following inoculation of the animals which received lecithin. An increase of the circulating monocytes in the control animals began at about the same time as in the experimental animals but lasted 2 to 3 weeks; that is, until about 5 weeks after inoculation. From then on, there were recurring peaks throughout the entire experimental period, so that actually 83 per cent of the 6 controls which were counted showed a fairly definite increase in monocytes. The findings in the controls are quite consistent with those classified by Cunningham, Sabin, Sugiyama and Kindwall (6) as group III, a group showing alternating periods of active and arrested stages of the infection.

Pathological studies: Comparisons of the amount of infection and healing in the treated and control animals of both series have been based largely upon the gross findings. While the blocks for sections were chosen from as representative areas as possible and are corroborative of the diagnosis and stage of healing in questionable instances, they represent merely a small area, and can be misleading as to the extent of involvement. In every instance the lungs of both experimental and control animals showed evidences of the reactions to infection, and presented stages of dissemination or healing which could be fairly accurately

analyzed at the time of autopsy. Early tubercles, necrosis, caseation, pneumonia, fresh scar-tissue and areas of old fibrosis were visible in all variations and mixtures. Involvements of the kidneys occurred in most of the animals of both series, and also of the testes in the second series, but these were much more difficult to evaluate, and all other organs were involved only sporadically and never extensively. Emphasis has therefore been placed largely upon the findings in the lungs. The grade of pulmonary involvement has been divided into four classes.

Class + represents practically healed tissues. In this class either no pulmonary tubercles at all were found, and no evidences of gross scarring, or else there were merely a few tiny dark scars without evidence of any central activity.

Class ++ represents lungs with very few, small and well-localized tubercles which were well walled and showed little surrounding pulmonary reaction. Usually only a few lobes contained such tubercles. The animals belonging to this class are regarded as individuals with actively healing pulmonary tuberculosis.

Class +++ represents a mild stage of pulmonary tuberculosis in which there were scattered tubercles usually in all lobes, but with evidences of a walling-off of the tuberculous areas and with normal white pulmonary tissue between them. The animals of this class would probably have eventually recovered in the absence of unexpected events, such as rupture of caseous areas with pneumonic extension.

Class ++++ includes animals with a generalized, massive pulmonary tuberculosis, with many areas of conglomerate tubercles, with active or beginning necrosis, with practically no evidences of healing and often with a phthisical pneumonia, as well as animals with a slightly less extensive involvement. In the latter case there were usually some uninvolved areas scattered between large foci of necrosis and caseation. All lobes showed tubercles, and there was only slight evidence of healing about any of the foci.

Table 1 gives the percentages of both experimental and control animals of both series which had pulmonary lesions falling into these four classifications. It also contrasts the total percentage of animals with actively healing or healed lesions (+ and ++) to those with questionably or very severe involvement (+++ and ++++).

The pulmonary involvements in both the control and experimental animals of series 1 were, in general, less than in the animals of series 2. A smaller percentage of the controls of series 1 had severe lesions than

of series 2 (63 per cent vs. 83 per cent). Correspondingly, a smaller percentage of the treated animals of series 1 had severe lesions than of series 2 (25 per cent vs. 46 per cent). The only difference between the experimental procedure employed for the controls of the two series was in the concentrations of the suspensions used for inoculation. It therefore seems probable that the generally greater pulmonary involvement of the animals of the second series was due to the greater concentration of the inoculum used for that series.

Irrespective of the more extensive pulmonary involvement of the animals of series 2, the effects from the treatment with lecithin were about the same in both series. Sixty-three per cent of the controls in series 1 had severe pulmonary lesions, while only 25 per cent of the treated

TABLE 1

Percentage of pulmonary involvements

GRADE OF PULMONARY INVOLVEMENT	SERIES 1 LECITHIN BEFORE AND AFTER INOCULATION WITH 720,000,000 BACILLI		SERIES 2 LECITHIN STARTED ONE WEEK AFTER INOCULATION WITH 1,640,000,000 BACILLI	
	Lecithin	Controls	Lecithin	Controls
+	58	14	27	4
++	17	23	27	13
Total	75	37	54	17
+++	17	36	34	48
++++	8	27	12	35
Total	25	63	46	83

animals had a similar grade of involvement. In the animals of series 2, 83 per cent of the controls had severe pulmonary lesions, while only 46 per cent of the treated animals had similar lesions. On the average, twice as many of the treated animals as of the controls of both series had mild or healing lesions. This means that the amount of healing in the treated animals was about 50 per cent greater than would have occurred without treatment.

In contrast to the findings in the lungs, no correlation can be established between the lesions of the kidneys and testes of the control and experimental animals. As in the case of the pulmonary lesions, the involvement of the kidneys and testes of the second series of animals was considerably more extensive than that of the first series. In fact, nearly every animal of the second series had lesions in those organs, and in many

instances there was extensive abscess-formation with complete obliteration of the normal structure of the testes and with much necrosis and scarring of the kidneys, together with great pockets of pus in the pelvices. On the other hand, the kidneys and testes of many of the animals of the first series appeared normal and the percentage of animals that had lesions in those organs is the same in the control and in the treated groups. The explanation for this lack of correlation between the treatment and the grade of involvement of the kidneys and testes is not evident. It seems possible that the lecithin may have been removed, or otherwise modified, before it reached the peripheral circulation going to the kidneys and testes. That this may have occurred in the infected pulmonary tissues seems probable. The phagocytic mononuclear cells which were present in the lungs in great numbers were obviously exposed to the lecithin before similar cells in other areas, and may well have acted upon it in a variety of ways. It is also probable that the locations of the individual tubercles in the lungs in relation to the capillaries may have made them more permeable to the circulating lecithin than were the tubercles in other areas.

The adrenals, mesenteric lymph nodes, spleen and liver were involved so infrequently in either series, and the involvement when present was so slight, that the findings in these organs seem irrelevant. One or more of these organs was slightly pathological in about 20 per cent of the animals of each group.

Studies of length of life: The percentage of animals of series 2 which died within various intervals of time after inoculation has been tabulated in table 2. The injections of lecithin in the experimental animals of this series were started a week after inoculation. Most of the deaths in the series were spontaneous, although a few animals of both the experimental and control groups were killed because of their physical condition. In addition, as has been discussed previously, a few of the experimental animals died immediately following injection. Many more of the control than of the experimental animals died within three months of inoculation. Actually, 47.8 per cent of the control animals had died spontaneously within three months of inoculation, while only 15.3 per cent of the experimental animals had died within the same period of time. Furthermore, one of the experimental animals (4 per cent) would probably have lived longer except for death consequent to an injection. After the interval of three months from inoculation, however, a greater percentage of experimental than of control animals

died in a given period of time. The average length of life of the control animals following inoculation was 115 days, and of the experimental animals, 148 days.

The decreased fatality of the experimental animals in the first few months following inoculation is in striking contrast to that of the controls, and may well be related to the lesser pathological involvement found in the lungs of the experimental than of the control animals. From the pathological findings, it seems probable that the early deaths in both the control and experimental groups were due largely to the pulmonary condition, while the later deaths were due to the involvement of the kidneys and testes. Most of the animals which died spontaneously

TABLE 2

Length of life in series 2

MONTHS SINCE INOCULATION	PERCENTAGE OF DEATHS	
	Lecithin	Controls
1	—	8.7
2	11.5	21.7
3	3.8	17.4
Total	15.3	47.8
4	19.3	8.7
5	19.3	8.7
6	11.5	8.7
7	19.3	17.4
Total	69.4	43.5
8	15.4	8.7

within the first few months of the inoculation showed a grade of pulmonary tuberculosis which has been classified as ++++ and exhibited few lesions elsewhere. On the other hand, many of the animals which died at later dates had the relatively slight grade of pulmonary involvement which has been classified as + and ++, but exhibited the massive infections of the kidneys and testes described above.

DISCUSSION

It has proved interesting to find that among the many remedies advocated from period to period in the treatment of tuberculosis, parenteral or buccal administration of various lipoids has received a conservative

encouragement for the past thirty years. The rationale for the treatment has been discussed from many angles, but has centered mostly upon indirect effects resulting from stimulation of the reticuloendothelial system by the lipoids.

The assumption of a stimulation to the reticuloendothelial system by the administration of lipoids was mainly a matter of theory, previous to the supravital studies of Sabin, Doan and Forkner (15) concerning the local reactions to injections of the lipoids from acid-fast bacilli, and of Tompkins (22), to injections of lecithin prepared from egg-yolk and of lipides made from brains. This theory was supported by a few reports of elevation in the number of circulating monocytes following injections of lipoids to normal animals, and by clinical observations of increases in the macrophages of the blood and tissues in diseases in which lipoids were known to be increased. Stassano and Billon (20) injected rabbits intravenously with lecithin and obtained a prolonged elevation of the circulating mononuclear cells, small ones appearing first, but followed later by large ones which, from the description, were probably monocytes. Bain (1) gave lecithin made from egg-yolk to rabbits both subcutaneously and by mouth, and obtained an increase in both red and white cells, the latter being dependent largely upon an increase in "large mononuclear lymphocytes." Fau (7) injected rabbits intravenously with water emulsions of the unsaponifiable fraction of lipoids extracted from various tissues, and within 24 hours of injection obtained rises in the circulating monocytes which lasted 4 to 5 days. Soula, Tapie and Fau (19) obtained the same findings with lipides extracted from splenic tissue. The studies of the blood in the experiments reported here reveal definite and maintained increases in circulating monocytes consequent to repeated intravenous injections of lecithin prepared from egg-yolk. The onset of the monocytosis shows a fairly consistent relationship to the interval of time which elapsed from the start of the injections and to the amount of lecithin given.

Practically all of the reports of the use of lipoids in the treatment of tuberculosis, either experimental or clinical, agree in the statement that the symptoms of the disease are ameliorated, and the progress is abated, but the infection itself is not eliminated specifically by the therapy. Claude and Zacky (5) found that the urinary excretion of phosphorus was less, and of nitrogen greater, in tuberculous guinea pigs given injections of lecithin in oil than in untreated controls. The treated animals lived longer than the controls, and were in a better state of nutrition at

the time of death. Treatment of human cases of pulmonary tuberculosis with the same substance given by mouth resulted in similar findings, and the beneficial effects of the treatment are considered as secondary to the effects on the nutrition as indicated by the excretion of nitrogen and phosphorus. Claude (4) found that, while the tissues of tuberculous guinea pigs treated with lecithin either by mouth or by injection were generally infected, they were less extremely so than those of the untreated controls and showed greater evidences of healing. Bain (1) discusses the use of lecithin by mouth in various clinical conditions, including tuberculosis, and attributes the beneficial effects therefrom to the stimulation to blood formation and to nutrition. He obtained an increase in the excretion of both phosphorus and nitrogen. Mattausch (11) (12) (13) made extensive studies of the treatment of human tuberculosis with various lipoids and obtained very satisfactory results, provided the procedure of treatment was modified on the basis of clinical details which he developed from extensive experience. He explains the beneficial results on the basis of the production of antibodies to fats and lipoids, and so to tubercle bacilli, by virtue of the therapeutic injections, and also on the basis of a stimulation to blood-formation and to a generally satisfactory state of nutrition. His studies include investigations of three different preparations; that is, *Yatren*, which is an organic compound of iodine said to stimulate mesenchymal tissue; *Lipatren*, which is a combination of *Yatren* and lecithin prepared from brain tissue; and *Gamelan* (called *Lipomykol* in Germany) which is a mixture of foreign waxes, lipoids and fats. Schlapper and von Opponkowski (16) obtained improvement in cases of human tuberculosis from the use of *Lipatren*, the results being better when the therapy was given parenterally than by mouth. Becker (2) also obtained good results by the use of *Lipatren* in carefully chosen cases, but undesirable effects when it was used indiscriminately. He attributes the beneficial effects to a stimulation of the mesenchymal cells by the *Yatren* component, and to the action of the lecithin component in stimulating body cells in general and blood and endocrine glands in particular, as well as in stimulating processes of immunization. Gewaltig (8) used *Lipomykol* both subcutaneously and intracutaneously in cases of pulmonary tuberculosis, and explains the beneficial effects which he obtained on the basis of stimulation of the reticuloendothelial system to a production of large mononuclear cells, to phagocytosis, to detoxication of substances liberated by the bacilli and to the production of antibodies toward lipoids. Kenner (9) likewise

found the same substance of value in cases of tuberculosis when it was given either subcutaneously or intracutaneously, and recommends its use in the home as well as in the clinic. Magat (10) treated tuberculous guinea pigs with *Helpin*, a mixture of lecithin in glycerine with various inorganic salts, and with a methyl-alcohol extract of tubercle bacilli; he found less extensive lesions in the treated animals than in the untreated controls. Siems (18) irradiated suspensions of lecithin in saline solutions of tricresol and tested the skin reaction of tuberculous patients to it. He obtained a more intense reaction than when a nonirradiated suspension of the same strength of lecithin was used. He theorizes that the beneficial effects that result from the treatment of tuberculous patients with ultraviolet light may be due to an effect of the rays on the lecithin which is normally present in the tissues, the effect being similar to that which he produced on the suspensions of lecithin in vitro. Scorpati (17) has recently published a preliminary report of favorable effects from the treatment of human cases of tuberculosis by intravenous injections of lecithin. The source of the lecithin is not stated.

From this survey of the literature, and from the results reported here, it seems evident that certain lipoids can exert an inhibitory influence on the course of a tuberculous infection, but do not in themselves act as germicides. Tubercle bacilli cause foci of infection wherever they lodge, but there is less extension of those foci and more rapid healing in the treated than in the untreated animals. The explanation for this effect from the use of lipoids is not certain. Considerable evidence points to the probability that the explanation lies in their ability to stimulate a production of macrophages in the connective tissues and thus indirectly to stimulate the various factors with which these cells combat foreign organisms. It is not evident how the lipoids stimulate a production of macrophages. The studies of Sabin, Doan and Forkner (15) and of Tompkins (22) show that, while different lipoids may act alike in initiating a production of macrophages, their ultimate influence on these cells may vary greatly, some causing evidences of increased physiological activity and some evidences of pathological activity leading to the formation of epithelioid cells. In view of these different ultimate effects, it seems likely that some lipoids would be far better therapeutic agents in the treatment of tuberculosis than others, and that some might even act detrimentally. It also seems probable that specific factors present in some lipoids rather than specific lipoids as a whole will be found to be the effective agents, and that the relative amounts of these factors will

determine the efficacy of the use of any particular lipoid in the treatment of tuberculosis. Practically all lipoids used thus far for that purpose have represented combinations of various lipoids, in which mixtures of lipides were undoubtedly a prominent component. Mattausch (12), in fact, considers mixed lipoids to be more effective than single components. It seems as if careful analysis of the effects of various pure lipoids and of their fractions may lead to extremely valuable information not only concerning the therapy of tuberculosis, but concerning many of the defense mechanisms to infection.

CONCLUSIONS

1. Repeated intravenous injection of rabbits with an emulsion of lecithin prepared from egg-yolk resulted in an increase in the number of circulating monocytes.

2. Intravenous injections of lecithin before and after intravenous inoculation of rabbits with bovine tubercle bacilli resulted in more rapid and extensive healing of the pulmonary lesions of the experimental than of the control animals.

3. Intravenous injections of lecithin, begun after intravenous inoculation of rabbits with bovine tubercle bacilli, resulted in a prolongation of life and a more complete and rapid healing of the pulmonary lesions of the experimental animals than of the control animals.

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THE MECHANICS AND BIOLOGY OF TUBERCULOUS CAVITIES[1, 2]

POL N. CORYLLOS

It is generally agreed to-day that tuberculous cavities represent the most important feature of pulmonary tuberculosis. They are not a mere complication of this disease or only an interesting pathological characteristic; they are the disease itself. It is because of cavities, said Laennec, that "tuberculosis may develop in any organ but phthisis develops only in the lung."

In fact they are the laboratories in which the tubercle bacillus lives and grows and its metabolic products develop, causing both local and general symptoms of phthisis. Chronic pulmonary tuberculosis without cavities is a quiescent and benign disease; with cavities it is a progressive and malignant one.

Therefore, since the 17th century, and more especially since the discovery of X-rays, which allowed the visualization and continuous observation of these cavities, all efforts have been directed toward finding ways and means by which their closure could be accomplished. Since the purely expectant treatment by bed-rest has been supplemented and gradually replaced by more modern and more effective surgical collapse methods such as artificial pneumothorax and, more especially, thoracoplasty, it has become increasingly interesting to know more about the mechanism of their closure, and of the relation of their closure to the clinical cure of the disease. It is obvious that more advanced knowledge on these points is of great help to our struggle against pulmonary tuberculosis.

The mode of production of cavities and *their pathology* are well known. Charcot and Richer (quoted by Grancher and Hutinel) have given, as early as 1886, an excellent description of them. According to these authors tuberculous lesions develop in the small bronchioli, the lumina of which become narrow and more or less obliterated by cellular proliferation.

[1] From the Department of Thoracic Surgery of Sea View and Metropolitan Hospitals of the City of New York and the Department of Surgical Research, Cornell Medical College.

[2] Presented at a session of the Clinical Section at the 31st annual meeting of the National Tuberculosis Association, Saranac Lake, New York, June 26, 1935.

Gradually caseous necrosis develops, the cells obstructing the bronchi are gradually liquefied, and the tuberculous exudate is emptied into the more distal bronchi. Thus, they say, by the "thawing" of the tubercles a cavity is formed connected with one or several bronchi.

A small cavity is thus formed which takes the place of the acini and bronchioles which disappear completely. The small vessels, arteries and veins are affected by endarteritis and thrombosis. These, "acinous cavities,"—continue Grancher and Hutinel, hang on the extremities of the healthy bronchi resembling terminal enlargements of these ducts. By progressive caseation these cavities increase in size and become lobular or even lobar.

Grancher, Rindfleisch and Rokitansky had already given, before 1885, a thorough description of the lesions of the bronchi which lead to the cavities; they have insisted upon the lesions of their walls, the irregularities of their mucosa and the changes of the diameter of their lumina. Often the bronchi were found dilated but more often they were narrowed or even obstructed.

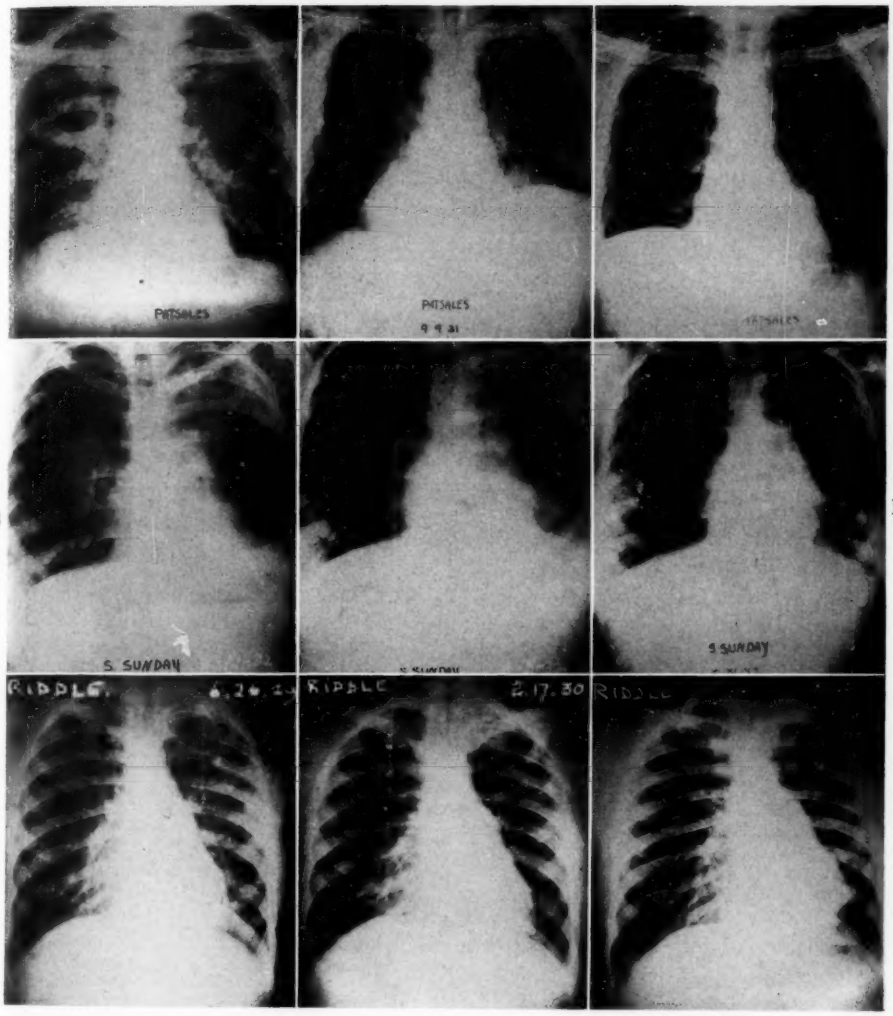
When we open a bronchus leading to a cavity, say Grancher and Hutinel, we see it suddenly open into the cavity without any transition; this opening cannot be distinguished from the walls of the cavity. As the cavity increases in size a greater number of bronchi open into it; Rindfleisch had shown that often, following the destruction of the small bronchi, the cavity opens laterally in a larger bronchus and appears to communicate with that bronchus through a hole drilled in the wall of the latter.

I know of no better or more comprehensive description of the formation of cavities; since that time little if anything has been added to the pathogensis of cavities. Laurell lately in an excellent radiological study has taken up this point and arrived at the same conclusions. Numerous exhaustive pathological studies have since corroborated this conception of formation of tuberculous cavities.

On the pathogenesis and mechanism of formation of cavities there is no discussion. This agreement, however, ends when we come to consider their evolution and more especially the mechanism of closure of the cavities; here we enter a ground of discussions and disagreement.

It is of interest to review rapidly the different opinions on these subjects. The majority of authors accept that increase in size of cavities is always due to the progressive destruction of the pulmonary parenchyma. Only very few phthisiologists seem to have noticed a number

Fig. 1A Fig. 1B Fig. 1C



Figs. 2A & 2B

Fig. 2C

Fig. 3A Fig. 3B Fig. 3C

Fig. 1. Spontaneous closure of cavity

G. P. White male. Large cavity in the middle field of right lung with diffuse infiltration around the cavity (A). Under bed-rest alone clinical symptoms disappeared, within 5 months (B). Sixteen months later (C) patient was well—actually working.

Fig. 2. Spontaneous closure of cavity

S. S. White male. Large cavity on left apex (A). Under bed-rest cavity disappeared within 3 months (B) and has remained closed (C).

Fig. 3. Closure and reopening of cavity

E. R. Colored female. Thin wall cavity of left apex. Cavity closed spontaneously (B). It has reopened, the same place, presenting the same shape a year later (C).

Fig. 4A Fig. 4B Fig. 4C

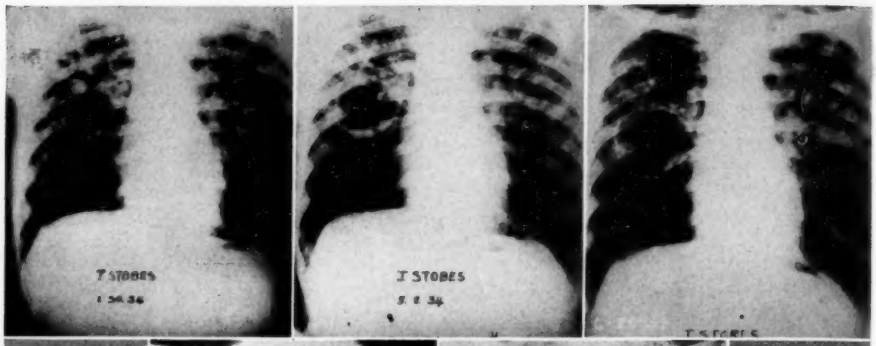


Fig. 4D

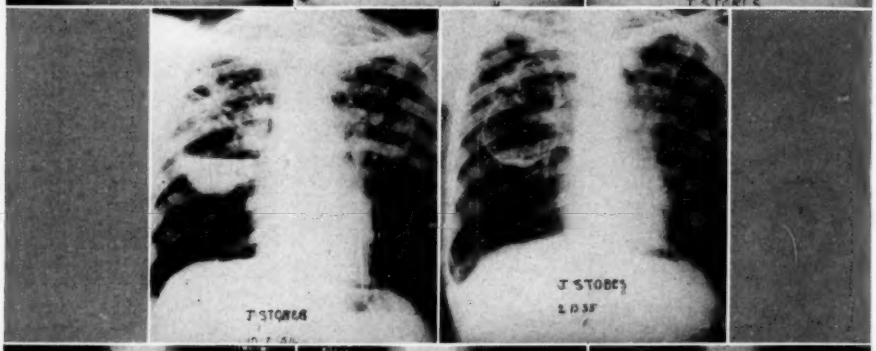


Fig. 4E

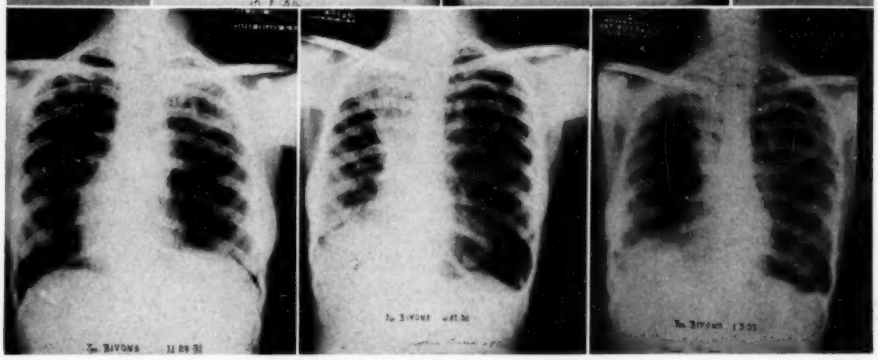


Fig. 5A Fig. 5B Fig. 5C

Fig. 4. Rapid change in shape of cavities. Check-valve cavities

T. S. Colored male. The period covered by the 5 X-rays is of 12 months' duration. The changes in shape, size without any apparent change of the walls of the cavities are characteristic. Notice retention of fluid in B and D; no fluid level in A, C, and E.

Fig. 5. Enlargement of cavity under pneumothorax, while contralateral cavity closes spontaneously.

T. B. White female. Bilateral cavities (A). Pneumothorax on left side produced satisfactory collapse of apex, only very thin adhesions were present. A year later the cavity on the side of pneumothorax had markedly increased in size (B and C) while the cavity on the right apex had almost disappeared. This difference in the evolution of two lesions on the same patient cannot be explained either on the grounds of general "resistance," "allergy" or "immunity" or of production of fibrous tissue or of elastic changes.

of peculiarities in the evolution of cavities which cannot be explained by this rather simple conception. In fact we all know that there are cavities which, after a period of progressive enlargement, suddenly stop enlarging and even decrease in size or disappear definitely (figures 1 and 2) or they reopen again later (figure 3). Cavities also often show very rapid enlargement as if they were inflated like balloons (figure 4). So far no definite explanations have been formulated about this peculiar behavior of cavities. The prevailing opinion is that the explanation of these phenomena should be looked for in the "development of fibrotic tissue and retraction of the diseased lung" or in "changes in the elasticity of the affected portions." It will be shown that these vague explanations displace the problem but do not solve it.

The discussion becomes more acute when the mechanism of healing of cavities is considered. Forlanini believed that cavities were formed by the wear and tear of the diseased parenchyma caused by the traumatizing effect of respiratory movements; therefore he considered as a necessary factor for healing of cavities to "put the lung to rest" by bed-rest, or by pneumothorax, in order to suppress the "traumatizing effect" of respiration upon the lung. A great many modern phthsiologists still adhere to this theory (Morelli, Monaldi, etc.).

Pinner advanced the theory that around the cavities two kinds of fibres are present; one set of concentric fibres acting as constrictors and tending to close the cavities, and a second set of radiating fibres, acting as dilators and tending to open them. Thus the size and configuration of the cavities would depend upon the relative strength and action of these two antagonistic systems of fibres. It is obvious that this ingenious theory is not supported by anatomical, pathological or clinical evidence. No such fibres were observed in histological examination; radiating fibres cannot dilate the cavities unless their ends distal to the cavity were solidly anchored to the chest-wall. Thus it became difficult to understand how cavities could remain open under pneumothorax, which deprives these fibres of any conceivable support, and even occasionally to increase in size (figure 5). Likewise, since scar-tissue once retracted cannot expand, reopening of cavities (as often occurs either spontaneously or following reëxpansion of the collapsed lung by pneumothorax) cannot be explained by the theory of Pinner.

More recently, Parodi in Italy, Dumarest and Lefevre in France, Bendove, and Van Allen and Wu in the United States endeavored to explain variation of the size and closure of cavities by changes of the elas-

ticity of the diseased lung. Parodi, in his first book (1928), advanced a peculiar mechanistic theory. He accepted the existence of two kinds of elasticity in the lung; one, elasticity of retraction which causes the lung to retract, and one, elasticity of expansion which causes it to expand. Furthermore, this author had endowed each of these two elasticities with the peculiar privilege of acting independently and even to be able to increase, decrease or disappear without influencing one another. Birnbaum and I have given elsewhere the reasons for which we oppose such a theory, as being against all mechanical, physical and physiological laws. Dumarest and Lefevre and Bendove have agreed with the theory of Parodi. Van Allen and Wu more recently have advanced a somewhat similar theory; they believe that closure of the cavities is due to "increased elasticity of the diseased parenchyma" caused by the "thickening of the interalveolar septa." But "increase of elastic strength" of a tissue can be produced only by the increase of its elastic elements; replacement of its elastic component by unelastic fibrous tissue, as is known to occur in pulmonary tuberculosis, not only cannot increase the elasticity but on the contrary decreases it and transforms the lung parenchyma into a rigid tissue. That the tuberculous lung retracts does not mean that it has lost its elasticity, and this fact was verified by us by direct measurements of the elastic tension of the tuberculous pulmonary tissue.

In his second book Parodi seems to have abandoned the above mentioned conceptions, and he attempts to explain closure of cavities and changes of their size by the action of the weight of the lung upon the diseased parenchyma. He supports this conception by involved mechanical considerations in which the lung is treated as a dead elastic body and in which contradictions are numerous. To cite only one example, the lung is in some places considered as one unit while in others the independence of its different lobes is stressed.

Monaldi in his recent excellent essay on the pathological physiology of the tuberculous lung accepts the conception of Morelli that the lung parenchyma "breaks at the point of the lesions; the 'breaking' tends constantly to enlarge because of the continuous tension of the elastic pulmonary parenchyma which is submitted to a static force, due to the negative intrapleural pressure, and to a dynamic force, due to respiratory movements." This traumatizing effect of respiration is more apparent, according to Monaldi, when a bronchus is stenosed because of the increased respiratory effort. Apical cavities, according to Morelli, "show

lesser tendency to enlarge because of the lesser motility of the apex as against cavities of the middle or lesser segments of the lung." For the same reasons, says Monaldi, the former does not present as marked changes in shape and size as the latter. On the other hand, according to these authors, in the apices, ulcerative processes are "less apt to progress" because of the limited movements of this segment which render the transfer of white cells and other cells to the lesion more difficult; thus the proliferative element becomes dominant. In the lower lobes, on the contrary, "greater motility favors the conveyance of pyogenic elements and consequently of ulceration."

It is difficult to accept these conceptions based exclusively upon the elasticity of the lung. I do not believe that the living lung can be considered merely as an elastic organ submitted exclusively to the mechanical laws of elasticity; it is obvious that the lung of a living patient is a living organ, the physiological functions of which are far more important than its elastic properties.

The above-mentioned authors have disregarded in their speculations the fundamental functions of the living lung; that is, gas exchanges and gas absorption. Moreover, they have, besides the physiology of the host, forgotten the physiology and metabolic characteristics of the guest, that is, the tubercle bacillus. It is of frequent occurrence in the history of medicine that the real aetiological factors have remained for a long while submerged under a mass of details of secondary importance; often the most extraordinary and fantastic theories have been elaborated in order to explain phenomena only in appearance puzzling and mysterious. But as soon as the true aetiological factors were discovered, the explanation of all phenomena became easy and clear.

CONCEPTION OF THE AUTHOR

In 1932 the author presented a new conception of the mechanics and biology of cavities. This conception has been accepted by many authors[3] and so far it has been refuted by none in print. Continued clinical and laboratory investigation on the subject since 1932 has rendered it possible to present in this paper a more complete report of this conception.

Let us follow the cavity from its inception. After the evacuation of

[3] Holcombe and Weber, Stivelman, Tapia, Jorda and Tapia, Ornstein and Ulmar, J. Martos, etc.

the caseated material a hole remains in the lung, communicating with the outside world by one or several bronchial outlets. (Figure 6.)

This hole does not collapse, as would occur in any other organ, because the lung contains air and because of the difference of pressure inside and outside of the lung. In fact, in the bronchi the pressure is equal to one atmosphere while outside of the lung the pressure is sub-

Fig. 6A

Fig. 6B

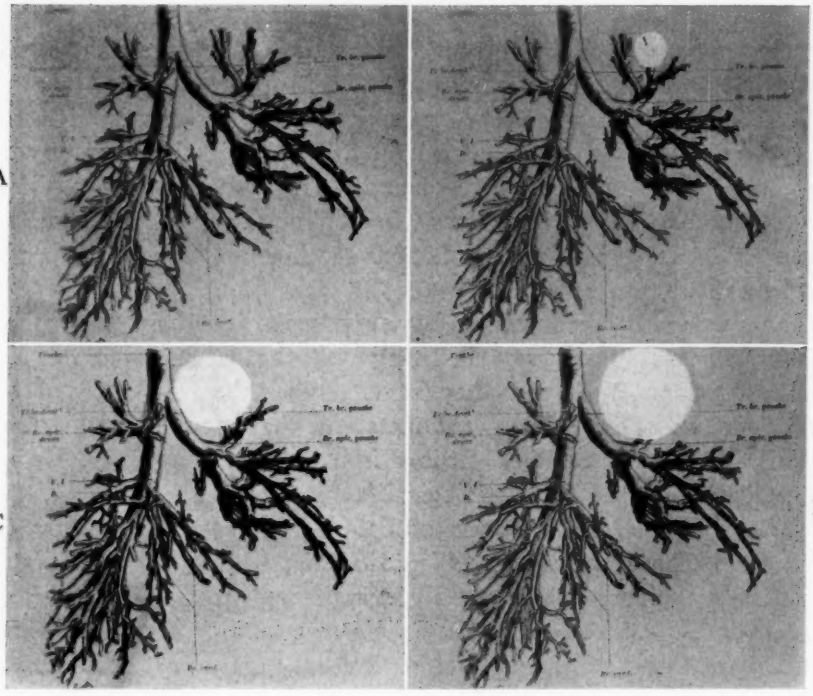


Fig. 6C

Fig. 6D

Fig. 6. Schematic presentation of formation of the bronchial outlets

(A) Bronchial tree. (B) Small cavity, away from the hilum, connected with one small bronchus. (C) Enlargement of the cavity results to its communication with two larger bronchi. (D) When the whole upper lobe is shelled out the cavity is directly connected with a lobar bronchus. Compare with figure 14D.

atmospheric and equal to the negative intrapleural pressure. For the same reason the cavity assumes a spherical shape in the living patient, while it appears irregular at the autopsy table, following the opening of the chest which suppresses the negative intrapleural pressure. Thus a cavity is spherical because the pressure inside of it is higher than around it, exactly as an abscess or cyst is spherical because it is filled with pus or fluid under tension.

The bronchial outlets of the cavities, also called "draining-" or "stem-bronchi," have been totally neglected so far. It is my belief that they represent the most important factors in the development of cavities. Anatomically they may be small or large, long and tortuous, or short and straight. Their size, length and shape depends on the degree of pathological change of the bronchi.

It is obvious that small peripheral cavities away from the large bronchus of the lobe will be most frequently connected with small bronchi (figure 6B). As the cavity increases in size by the progressive destruction of the pulmonary parenchyma larger bronchi will open into it; when a whole lobe has sloughed out the lobar bronchus will open directly into the cavity (figure 6D).

The draining bronchi are not hypothetical features. Long ago they were seen in radiograms (figures 7 & 8) and pathological specimens (figure 9) and were reported by many authors. They are especially well visualized when lipiodol is injected into the cavity through the chest-wall, as was well shown by Ameuille in 1924 (figure 10).

Having established the anatomical characters of these draining bronchi, we can study their physiological functions. It seems reasonable to admit that changes in the lumina of the bronchi, and especially their closure, must necessarily influence the further evolution of cavities. It is well known that when air is entrapped anywhere in our tissues, be it the subcutaneous tissue, the peritoneum, the ventricles of the brain, the pleura, and more especially the lung, it is finally absorbed by the circulating blood. Obstruction of a pulmonary bronchus is always followed by absorption of the air contained in the corresponding portion of the lung and atelectasis is produced.

On the other hand pulmonary tuberculosis begins in the bronchi, which present marked lesions early in the disease. These lesions may be allergic, ulcerative or proliferative. All these lesions produce the same mechanical changes, namely narrowing of the lumina of the bronchi; caseation of the mucosa and of the bronchial wall may sometimes produce enlargement, but most often they cause narrowing and obstruction which may be temporary or permanent.

Thus three eventualities may occur: (1) the bronchial outlets of a cavity may remain open; (2) they may close; or (3) they may become narrowed. In the last case a one-way valve mechanism may develop, as in foreign bodies or tumors of the bronchi, which allows ingress of air during inspiratory expansion of the bronchi, whereas during expiratory

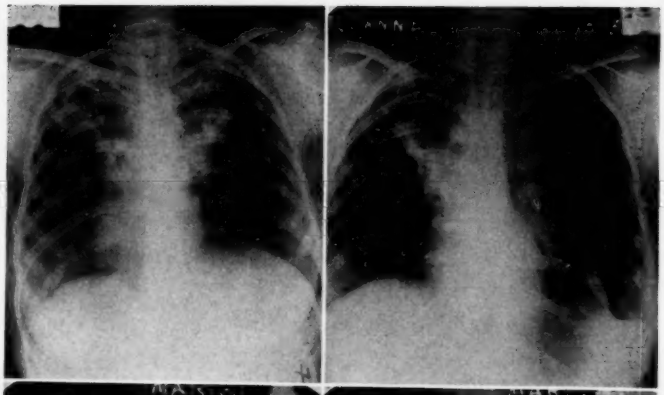


Fig. 7A

Fig. 7B

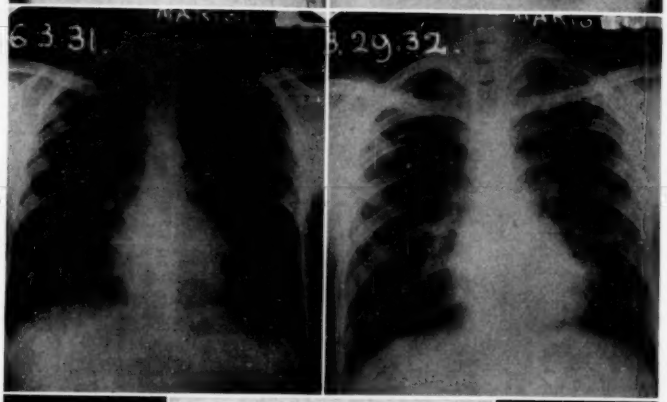


Fig. 8A

Fig. 8B

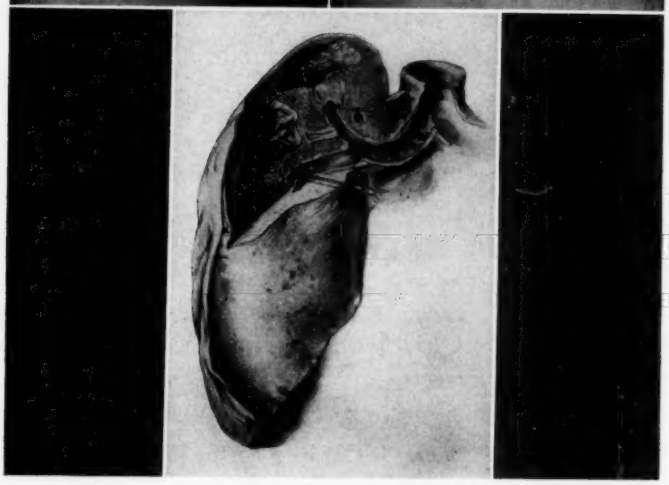


Fig. 9

Fig. 7. Stem-cavities

M. R. White female. Bilateral stem-cavities. The tennis racquet shaped cavities and their draining bronchi, especially on the left side (A) show very well. Pneumothorax on that side has rendered more visible the draining bronchus (B).

Fig. 8. Stem-cavity

A. M. White female. Stem-cavity left apex (A). Notice the enlargement of cavity although patient was on strict bed rest, and although it has elastic walls (B).

Fig. 9. Drawing of specimen showing the draining bronchi in which probes were introduced.

Fig. 10A Fig. 10B Fig. 10C

Fig. 10. Lipiodol injection into a cavity through the chest wall (Radiograms communicated by Dr. Ameuille). (A) X-ray taken during injection of lipiodol. Needle still in place. Notice lipiodol in the cavity and in the draining bronchus which is beautifully outlined, reaching the large bronchus. (B) 5 minutes later, lipiodol begins to penetrate into the other bronchi. (C) 15 minutes after injection. Lipiodol has penetrated in lower bronchi producing what Ameuille terms "bronchial embolism."

collapse of the same bronchi obstruction becomes complete and egress of air is prevented.

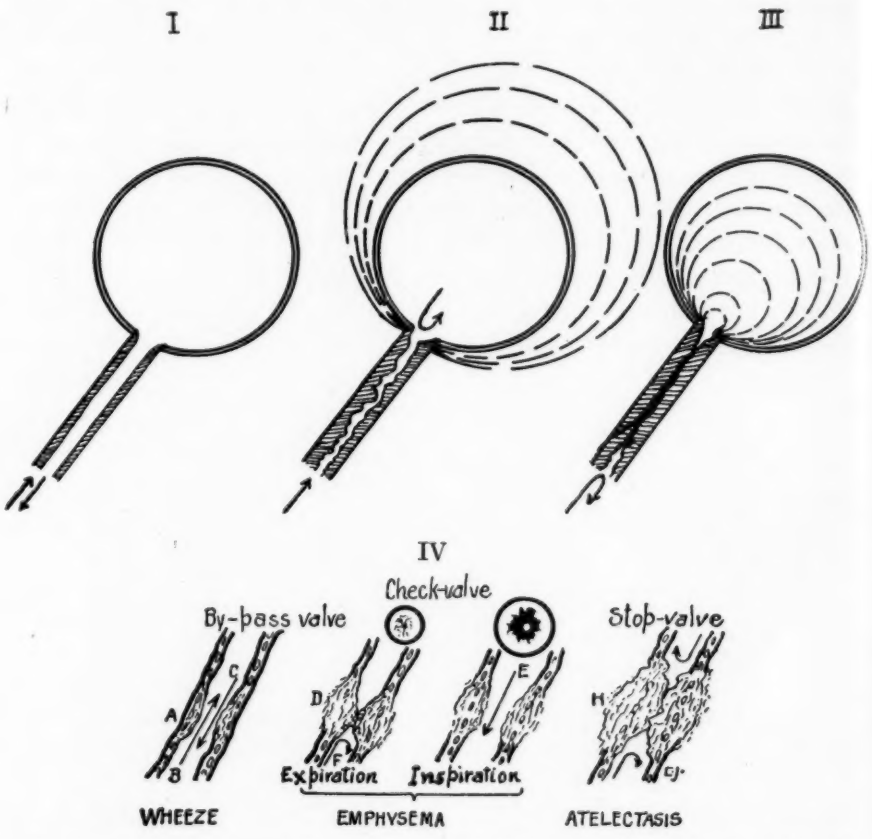


Fig. 11. Schematic presentation of the physiological action of draining bronchi. (I) Bronchus open; ingress and egress of air free. No other changes occur in the cavity than the ones caused by progressive sloughing. (II) Check-valve cavity. Allergic inflammatory or proliferative narrowing of bronchus may produce one way valve mechanism. Ingress of air can take place while egress is hampered. Obstruction emphysema is produced causing ballooning of cavity. (III) Closure of bronchus, causes "atelectasis," that is, absorption of the air entrapped in the cavity and shrinkage and disappearance of cavity, without any conspicuous scar. For the composition of gases and pressures see text. (IV) Jackson's explanation of the production of the phenomena under similar conditions in cancer of the lung.

In the first case when the bronchi remain open (figure 11, I), air will circulate freely in and out of the cavity, ensuring, as we shall see, the unhampered growth of the tubercle bacilli and draining the content of the cavity into the bronchi. In these cases bacilli are present in the

sputum and the products of the bacilli are freely absorbed by the lymphatics of the lung. Thus the usual expression of "open tuberculosis" acquires a real clinical and pathological meaning.

In the second eventuality (fig. 11, III), that is, if the draining bronchus of the cavity becomes obstructed, a series of important phenomena are apt to occur. First, the severance of communication of the cavity with the bronchial tree will naturally cause the disappearance of tubercle bacilli in the sputum. This explains the thus far unaccounted-for phenomenon of the sputum becoming negative, sometimes spontaneously, more frequently shortly after induction of pneumothorax or following thoracoplasty, although the cavity may still remain visible in the radiogram. Furthermore, the closure of the bronchus will cause, necessarily, the absorption of the gaseous content of the cavity by the mechanism

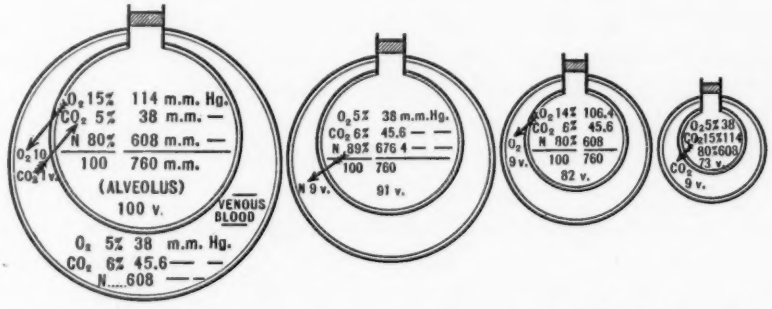


Fig. 12. Intimate mechanism of gas absorption in experimental atelectasis as shown by Coryllos and Birnbaum. Notice the similarity of these phenomena with closure of tuberculous cavities following obstruction of the draining bronchi.

described elsewhere (figure 12). The speed of gas absorption will be regulated by the degree of circulation around the cavity; it will be very rapid in small cavities with little infiltration and fibrosis around the cavities, in other words in the so-called young and elastic cavities. It will be slow in large and thick-walled cavities, or become impossible when a whole lobe is shelled out leaving no pulmonary parenchyma around it. As the absorption of air progresses the cavity will shrink and finally it will disappear, leaving little or no traces whatever. The cavity has disappeared simply because its gaseous content was absorbed; atelectasis has developed in it. If for any reason the draining bronchus reopens it is obvious that the cavity will reappear. This simple theory explains the peculiar changes of the size of cavities (fig. 5), the occasional retention of the fluid in them (figure 4B and D), and the para-

doxically often good prognostic significance of the appearance of these fluid levels in the cavity. This conception explains the beneficial effect of bed-rest, which decreases the metabolic needs for oxygen of the organism and consequently decreases the amount of the tidal air and of the respiratory expansion of the diseased bronchi. Application of weights upon the diseased apex, which was empirically found to be helpful, acts by still decreasing thoracic amplification and consequently the inspiratory expansion of the bronchi of the affected areas. Phrenicectomy, pneumothorax, closed pneumolysis and thoracoplasty act by the same fundamental mechanism as bed-rest, in favoring the collapse and closure of the bronchial outlets of the cavities. This is the mechanism, common to all medical or surgical methods of treatment of pulmonary tuberculosis, by which "rest" of the lung brings about arrest and cure of the disease.

The third eventuality (figure 11, II) is narrowing of the draining bronchus and formation of a check-valve mechanism. In these cases, as in similar conditions produced by foreign bodies or tumors or even by functional narrowing of the bronchi as in bronchial asthma, or anaphylactic shock in the guinea pig, obstructive emphysema is produced beyond the bronchial obstruction, as Jackson and Manges have showed many years ago (figure 11, IV). The cavity increases in size within a short time and without any marked changes in its wall. Because of narrowing and expiratory obstruction of the bronchial outlets, the cavity is "ballooned." According to the course of bronchial narrowing the "ballooned check-valve cavity" may remain so, or it may close completely if the bronchi become obstructed, or it may revert to its previous size if the bronchus has reopened allowing free egress of air.

Biological Effects of Closure of Draining Bronchi: Still more important are the biological effects of the closure of draining bronchi upon the tubercle bacilli contained in the cavity. Novy and Soule and more recently Loebel, Shorr and Richardson have shown in a thorough investigation that the human tubercle bacillus is a strict aerobe; suppression of oxygen for a few days only causes its respiratory metabolism to fall to zero, and strongly injures or even kills the germ. These authors have shown (1933) that "two days of anaerobiosis of tubercle bacilli in Long's Solution led to destruction of two-thirds of their respiratory capacity, and 3 or 4 days of anaerobiosis to complete abolition of respiratory metabolism; corresponding to this high grade of damage to respiration

there was failure to grow on Long's medium when transplants were made at the end of the anaerobic period." In the above experiments Loebel, Shorr and Richardson produced complete anaerobiosis by replacing the air in the Warbourg cells, in which H37 cultures were placed, with nitrogen. But even when O_2 was present, provided that its tension was not higher than 0.2 per cent, growth was inhibited, and when the cultures had remained over 35 days in a pressure of O_2 of 0.19 (or if the organisms were allowed to exhaust the air in a sealed tube) no growth could be obtained after readmission of air. To the same conclusions had arrived Novy and Soule (1925), Corper, Lurie (1927) and Uyei. These experimental conditions of anaerobiosis are produced in a tuberculous cavity following occlusion of its bronchial outlets. In fact oxygen is actively absorbed both by the circulating blood and the tissues and cells present in the cavities. On the other hand arrest of growth stops the formation of toxic products. This explains the rapid improvement of the patient following closure of the bronchi of the cavities. It also explains the improvement of contralateral lesions by the suppression of perifocal infiltrations due to tuberculin which is formed in considerable amounts in the large cavities.

It seems advisable to further develop here this question of asphyxiation of tubercle bacillus, which, although of such tremendous importance to the phthsiologists, is generally ignored or neglected by them.

It can be said that what we mean by telling that "nature tends to cure tuberculosis" can be translated in scientific language, that "all tissues of the lung and more especially the 'monocytes,' which aggregate around tubercle bacilli forming the anatomical tubercle, tend to absorb oxygen and thus asphyxiate tubercle bacilli."

Loebel, Shorr and Richardson endeavored to calculate what dimensions a tubercle would have to reach in order to reduce the oxygen supply in its centre to zero. It is evident that these calculations apply as well to tubercle bacilli present in the cavities following occlusion of their bronchial outlets as to lymphnodes or tubercles.

If we assume that a tubercle is a sphere the equation for that calculation reads

$$R' = \sqrt{6Co\,\frac{D}{A}}$$

Where R' = the radius in centimetres
Co = the pressure of O_2 in mm. of Hg
D = Krogh's coefficient of diffusion of O_2 at 20°C. (1.4×10^{-5} cc. per square centimetre).
A = the oxygen consumption in cubic centimetres per moist gram of tubercle-bacillus culture per minute

By applying Schlossmann's value of 13 cc. for the oxygen consumption per dry milligram of culture per hour and Campbell's (1931) lowest figure for oxygen pressure under the skin 25 mm. Hg, one obtains from the above equation 0.075 mm. as the critical thickness of the

tubercle. In other words in the center of a tubercle the diameter of which is greater than 0.075 mm. no O_2 will be present. Even if we use the lowest figures obtained for the oxygen consumption, 2.4 cm. per dry milligram per hour and the highest figures for the oxygen tension, namely, 100 mm. Hg, which is that of the arterialized blood in the lungs, the calculations for the critical thickness of the tubercle will yield the value of 0.35 mm.

From these figures, it is readily seen that it would not require very large aggregates of cells to produce a condition of oxygen want inhibiting the development of tubercle bacilli while the tissue cells will be able to survive. The cause of this extremely important phenomenon is that the cells have an alternative source of energy; they can decompose glycose to lactic acid which tubercle bacilli are unable to do.

It has been shown (Okamoto, 1925) that carcinoma cells will be kept alive by this lactic-acid mechanism and can be transplanted successfully after 3 days of anaerobiosis. White cells of the rabbit and of the horse conserve their amoeboid movements after 12 hours of asphyxiation by means of cyanide.

On the contrary the tubercle bacillus cannot derive energy by the lactic-acid mechanism. As Soule junior put it, they do not possess the necessary enzyme—"anaerase"—by which anaerobes can live by anaerobic processes.

From the facts presented, conclude Loebel, Schorr and Richardson, conditions develop in the tissues affected with tuberculosis "which produce oxygen lack or even anaerobiosis which may serve to check and perhaps to destroy the bacilli which are present in the lesion." In the specific case of human tubercle bacillus, as H37, according to the same authors "anaerobic exposure on Long's medium leads to a complete destruction of both respiratory and proliferative capacity in a few days."

Another, no less important, biological effect of closure of the draining bronchi and atelectatic shrinkage of cavities and of the surrounding pulmonary parenchyma is the development of fibrous tissue. This is due to the anoxaemia of the diseased lung parenchyma caused by atelectasis which suppresses both ventilation and circulation of the alveoli. Tissue anoxaemia when sufficiently pronounced and prolonged leads to fibrosis. The so-called ischaemic paralysis of Volkmann is a well known example. It is precisely for this reason that fibrosis in the tuberculous lung is selective and limited to the diseased portions. As a rule, when a lung which had been collapsed by pneumothorax for a long time is allowed to reëxpand, only the healthy portions of it do reëxpand. There is nothing puzzling about this phenomenon. In fact, the lung has a dual circulation: functional through the pulmonary arteries and veins which takes very little part in the nutrition of the pulmonary parenchyma and a nutrient circulation through the bronchial arteries and veins which takes very small part in gas exchanges. Thus, fibrosis does not develop in the healthy parenchyma in which the nutrient circulation remains unchanged and is not affected by the collapse of the lung; on the contrary, in the diseased parenchyma where lesions of the arterioles of both circulatory systems and especially of the bronchial one are caused by

the disease, ischaemia becomes marked and permanent, and leads to fibrosis or to necrosis according to its degree.

Thus, the cycle of events leading to the arrest of the disease is complete; closure of bronchial outlets, disappearance of cavities, negative sputum, arrest of development of toxic products with clinical improvement of the patient and fibrosis of the lung. The links of this chain are solidly riveted, and none of them can be suppressed without destroying the succession of events. All phenomena occurring in the course of the disease are thus clearly explained by simple physiological, mechanical, physiochemical and bacteriological facts which are well known and firmly established. There is no need for involved or metaphysical theories.

Furthermore, this conception establishes the *identity* of the mechanism of action of bed-rest, pneumothorax and thoracoplasty, all of which promote clinical cure of pulmonary tuberculosis by slowing down pulmonary ventilation and thus decreasing or suppressing the oxygen necessary for the continuation of life and growth of tubercle bacilli; it gives a clear and physiological explanation of the changes whereby an "open" and an active lesion becomes "closed" and inactive, akin to small tubercles or old tuberculous lymph nodes following a primary infection, which will remain inactive so long as the fibrous shell of tubercle remains closed. But if and when this fibrous shell is disrupted the chronic and arrested lesion again becomes active, simply because the tubercle bacilli which have resisted anoxaemia and have remained alive find again a favorable environment for growth and development. The same thing will happen to a closed cavity if its bronchial outlets open again.

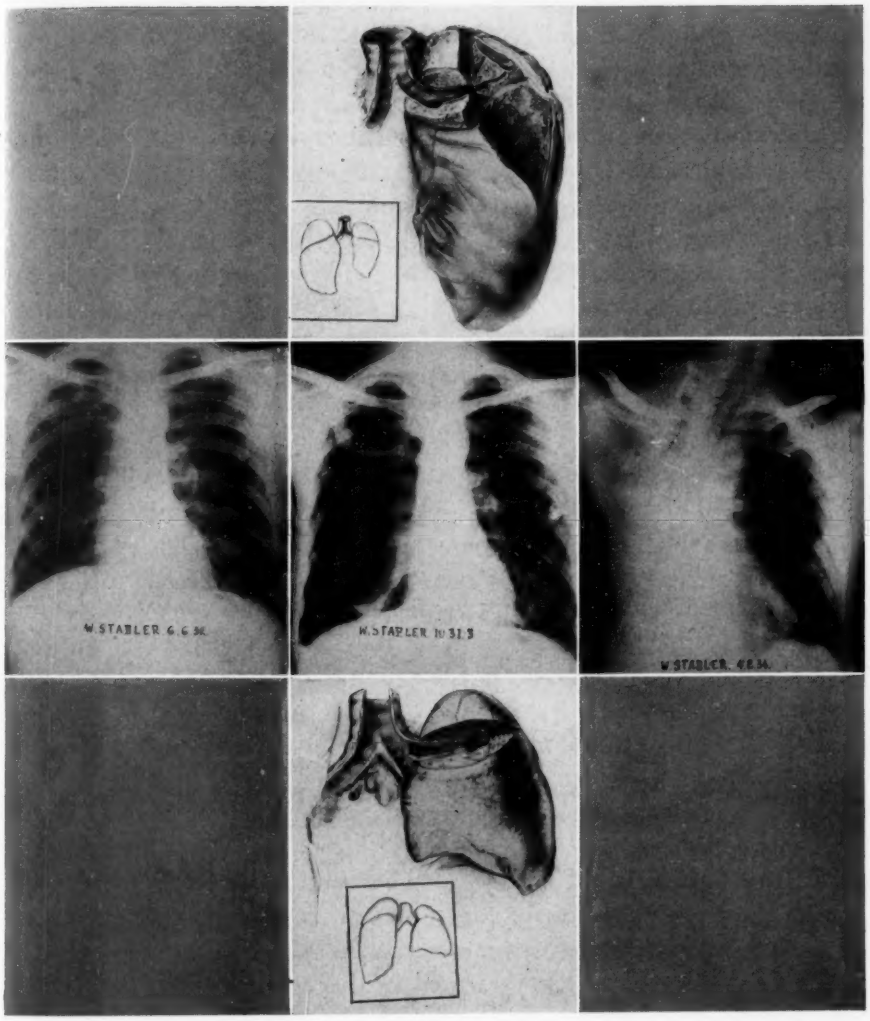
Physical Evidence: The above developed conception upon the importance of the bronchial outlets of tuberculous cavities was further corroborated by our investigation on the changes of the chemical composition of the air contained in the cavities, and of the pressures and manometric oscillations in them (figure 11). The technique used in this study consisted in introducing a 20 gage needle through the chest-wall into the cavity. The results obtained were as follows: In cavities with open bronchi (patients with active disease, highly positive sputum and no fluid-level in the cavities) the percentages of O_2 were around 16–19 per cent, while CO_2 was less than 1 per cent; the pressure was equal to atmospheric and the oscillations of -1, $+1$ mm. Hg, that is, comparable

to the oscillations of the bronchial air. These findings prove clearly that a free ingress and egress of the outside air was going on in the cavity as shown by the high content of O_2 and low percentage of CO_2 (figure 11, I); furthermore that the bronchial outlets of the cavities were open as shown by the atmospheric pressure in the cavity and its oscillations. The findings were different in the ballooned (check-valve) cavities; the oxygen content in these cavities was around 10–17 per cent, the CO_2 around 2–3 per cent; the pressures were always positive (+2 to +4) and there were no oscillations or very weak and only inspiratory ones. These findings show that the air was entrapped in the cavities so that O_2 was partially absorbed and less than in the alveolar air; but also it was partially renewed because CO_2 remained lower than in the alveolar air; also that the bronchial outlets were closed during expiration because the pressures were higher than in the bronchial tree and there were no expiratory oscillations. The frequent presence of a fluid-level showed the defective drainage through the narrowed bronchial outlets. On the contrary, in closed cavities (figure 11, III), especially following thoracoplasty it was particularly impressing to find the percentage of oxygen falling within a few hours below 1 per cent, or even disappearing completely, whereas the percentage of CO_2 was becoming almost as high as in closed pneumothorax with exudate, that is, above 5 per cent. The pressures were always negative and oscillations were absent.

Pathological Evidence: Besides the clinical and experimental data presented above which are all in favor of this conception, we had a number of postmortem specimens which have further corroborated this theory. Only two cases will be reported here. One is a case of a white female of 40. She died after the second stage of thoracoplasty which succeeded in closing a large thick-walled apical cavity. The sputum had been constantly negative following thoracoplasty. In the autopsy specimen (figure 13) the cavity is represented as a mere slit and its two bronchial outlets are perfectly closed.

The other case is of a white male of 45 who had a huge right apical cavity with very thick walls which had resisted prolonged pneumothorax. A three-stage thoracoplasty, followed by anterolateral thoracoplasty and extrapleural packing, had failed to suppress the bacilli from the sputum (figure 14). This patient died 7 months after the last stage with strong contralateral emphysema and decompensated heart. In the specimen we found that the upper lobe was shelled out and the cavity

Fig. 13



Figs. 14A & 14B

Fig. 14C

Fig. 14D

Fig. 13. M. P. White female. Clinical cure following thoracoplasty. Sputum persistently negative. Patient died 18 months later. Notice collapse of a thick wall cavity; the bronchi leading to the cavity were closed.

Fig. 14. White male. Large cavity 5 x 6 inches (A). Pneumothorax was without effect upon the cavity (B). Three stage thoracoplasty and packing failed to collapse the cavity. Although a good mechanical collapse was obtained (C) sputum was persistently positive. Death 7 months later. Autopsy showed huge uncollapsed cavity connected with a large open lobar bronchus.

was connected with the lobar bronchus which had remained open (figure 14D).

I believe that the clinical, experimental and pathological data presented here cannot leave any doubt upon the paramount importance of the bronchial outlets of tuberculous cavities in the evolution and the fate of the latter. The mechanism of the changes occurring in these cavities from their formation to their closure is regulated by the physiological functions of gas exchanges and gas absorption of the lung. The cure of the disease, or rather its change from progressive and active to arrested and inactive, is based upon the biological needs of the tubercle bacillus of large amounts of oxygen for life and growth. Last, the development of fibrosis in the diseased lung is simply caused by the anoxaemia which follows atelectasis and ischaemia of the diseased parenchyma.

CONCLUSIONS

1. The different theories so far presented for the development and closure of cavities are discussed.

2. It is shown that none of them is able to explain all the peculiar and so far unaccounted for changes occurring in tuberculous cavities.

3. The anatomical, mechanical, physiological and biological importance of the bronchial outlets of the cavities are shown. The conception of the author upon their rôle in the evolution of tuberculous cavities is exposed.

4. It is shown that *all* changes occurring in tuberculous cavities can be easily explained by that conception.

5. Anatomical, physiological biological data are presented in favor of this conception.

6. Investigation of the changes of the chemical composition of the air contained in the cavities, of pressures and respiratory oscillations in them, are again in favor of this conception.

7. Pathological data are presented further corroborating the theory of the author.

8. The division of cavities into cavities with open, closed or narrowed (forming check-valve) bronchial outlets is supported by anatomical, radiographic, physiological, biological, experimental and pathological evidence.

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THE TEACHING OF TUBERCULOSIS[1]

At the University of California Medical School

E. ROSENCRANTZ[2]

The teaching of tuberculosis in the University of California Medical School is done almost entirely at the San Francisco Hospital. There the University controls a service of 190 available beds—160 for adults (120 men and 40 women), and 30 for children. Since it is a municipal hospital, it has patients in all stages of the disease; the majority of the adults, however, are far advanced.

OUTLINE OF TEACHING IN TUBERCULOSIS

A: Second Year (San Francisco Hospital)
Second semester
Physical diagnosis

B: Third Year (San Francisco Hospital)
First semester
Lectures and clinical demonstrations—9 hours
Ward work—13 days
Second semester
Ward work and special procedures—2 weeks

C: Fourth Year (University of California Hospital)
General medical clinic—Out-patient Department—10 weeks
Consultation on tuberculosis cases with Chest Clinic Chief

D: Interne Year
Hospital—Tuberculosis Service (San Francisco Hospital)—2 months
Chest Clinic—4 nights
Sanatorium—(Hassler Health Home)—1 month

[1] Read at the meeting of the Association of American Medical Colleges, October 14, 15, 16, 1930.

[2] University of California Medical School, Division of Medicine, San Francisco, California.

A: Second Year

During the spring semester of the second year, the students have physical diagnosis three times a week—one hour's lecture followed by two hours of work in the wards. The time allotted to the lungs is 13 mornings—5 for the normal, and 8 for the abnormal.

The class is divided into sections of 8 students. For normal lungs the students examine each other. The physical signs of abnormal lungs are taught mainly on patients in the tuberculosis wards. Each instructor takes his group to the ward assigned to him, but any rare or strikingly abnormal chest throughout the service is seen by the entire class.

During these mornings, aside from learning the fundamentals of physical diagnosis, the students also learn the physical signs of the various chest diseases such as pleurisy (acute, fibrous), effusion, spontaneous pneumothorax, hydropneumothorax, pyopneumothorax, bronchitis, bronchiectasis, emphysema, various forms of tuberculosis, abscess, tumors of the lung and pleurae, pneumonia, asthma, and fibroid lungs.

B: Third Year

The work during this year consists of lectures, clinical demonstrations, ward- and class-work. The following section describes this in detail.

First Semester

Lectures and Clinical Demonstrations

I: General factors:

1: History of Tuberculosis: "aeroplane flight" through the centuries from Hippocrates to Calmette

2: Paths of infection
- (a) Inoculation
- (b) Inhalation
- (c) Ingestion

3: Zoological distribution
(natural)

4: Predisposition (local)
(acquired)

II: Classification of tuberculosis

1: Miliary tuberculosis
- (a) Typhoid form
- (b) Acute pulmonary form
- (c) Meningeal form

Each form discussed according to
- Pathology: specimens shown
- Clinical course
- Laboratory findings
- X-ray films
- Differential diagnosis

} Patients demonstrated when available

III: Lymphatic tuberculosis

1: Glandular
- (a) Generalized—specimens
- (b) Local
- (c) Tabes mesenterica—specimens

} Demonstration of patients, charts, films

2: Serous membranes
- (a) Polyorrhomenitis
- (b) Pleura
- (c) Pericardium
- (d) Peritoneum

} Taken up in detail in ward. (Patients shown when available.)

IV: Pulmonary tuberculosis

1: Caseous pneumonia

(a) Pathology; symptoms; signs; clinical course; laboratory findings; X-ray films; differential diagnosis. (Patients shown when available.)

2: Chronic ulcerative tuberculosis
- (a) Onsets
- (b) Symptoms
  - Local
  - General

} Mentioned for the continuity of the subject. They are taken up in detail in the ward work.
- (c) Complications

Complications studied in detail according to system

V: Treatment

1: Natural healing
- (a) Clinical evidence
- (b) Anatomical evidence

2: Public prophylaxis
- (a) Fundamental factors
  - Destruction of the tubercle bacillus
  - Building up of resistance
- (b) State and municipal laws
  - (1) Compulsory notification
  - (2) Prohibition of expectoration in public places
  - (3) Dairy and milk inspection
  - (4) Cattle testing; slaughter-house inspection

(5) Regulation of trades, workshops, home industries (slides)
(6) Tenement house inspection (slides)
(7) Board-of-health activities; disinfection

(c) Construction and establishment of
(1) Health centres
(2) Dispensaries
(3) Sanatoria; hospitals
(4) Preventoria
(5) Open air schools
(6) Day camps
(7) Farm colonies
} Slides

(d) Education
(1) Popular lectures
(2) Health program; tuberculosis week; instruction in school; tuberculosis sermons
(3) Sale of tuberculosis seals
(4) Inspection of school children
(5) Examination of high-school children: tuberculin tests, X-ray films of chest; motor clinic to rural districts

(e) Anti-tuberculosis campaign
(1) National, state, county, tuberculosis associations
(2) Sale of tuberculosis Christmas seals
(3) Bureau of Tuberculosis in State Board of Health
(4) Standardization of the county tuberculosis hospitals and sanatoria for the state subsidy
(5) Examination of the workers in various industries—recommendations and follow-up work
(6) Social service work and the visiting nurse

(f) Institutional care
(1) Preventoria
(2) Sanatoria
(3) Hospitals
(4) Farm colonies
(5) Day colonies
} Illustrated by slides showing views of institutions in Germany, France, Switzerland, and England, as well as those at home.

3: Medical
(a) General regimen
(b) General medication
(c) Special medication
(1) Local symptoms
(2) General symptoms
(3) Complications
(d) Diet
(e) Bowels

4: Specific
- (a) Tuberculin
  - (1) History
  - (2) Diagnostic tests, animal and man
  - (3) Treatment
- (b) Surgery
  - (1) Pneumothorax
  - (2) Phrenectomy
  - (3) Pneumolysis
  - (4) Thoracoplasty

  } Films, Patients
- (c) Artificial-light therapy
- (d) Rollier treatment (slides)
- (e) *BCG* treatment (Calmette-Guérin)

Ward and Class Work

The topics taken up in detailed study in wards and class are as follows:

A: Survey of cases in ward
B: Onsets of chronic pulmonary tuberculosis
C: Local symptoms of pulmonary tuberculosis
D: General symptoms of pulmonary tuberculosis
E: Miliary tuberculosis
F: Pulmonary tuberculosis
- *I:* Acute caseous
- *II:* Chronic pulmonary
  - *1:* Incipient
  - *2:* Moderately advanced
  - *3:* Advanced
- *III:* Complications (According to Systems)
  - *1:* Respiratory
    - (a) Larynx
    - (b) Pleura
      - (1) Acute
      - (2) Fibrous pleurisy
      - (3) Effusions
      - (4) Spontaneous pneumothorax
      - (5) Hydropneumothorax
      - (6) Pyopneumothorax
    - (c) Lungs
      - (1) Hemoptysis
      - (2) Compensatory emphysema
      - (3) Subacute disseminated

2: Gastrointestinal tract
3: Skin
4: Lymphatic
5: Locomotor } In detail in second semester
6: Urinary }
G: Fibroid tuberculosis
H: Nontuberculous pulmonary disease,
Pneumonia, abscess, empyema,
Bronchitis, emphysema, asthma,
Bronchiectasis, pneumonoconiosis, } Differential diagnosis
atelectasis, neoplasm

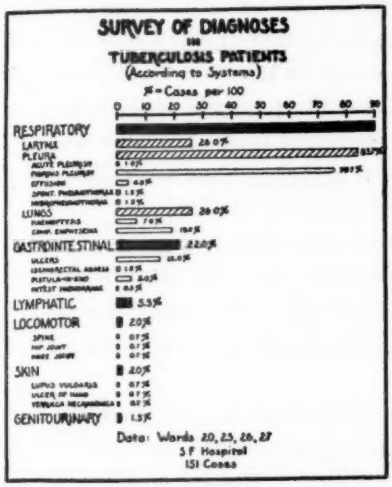
During the first semester, the class is divided into sections of ten students. Each section remains with us for thirteen days. A schedule and outline of the work for this time is given. The students work in pairs in the ward assigned to them. A special study is made of the subjects as indicated above, with physical examination of the chest, and a graphic chart made of the findings.

A: Survey: Each student makes a list of the patients in his ward with the number of the bed and all the diagnoses (which he finds on the first page of the history). In class in the afternoon, the students meet the instructor and together they discuss the diagnoses; that is, tuberculosis with the complications according to systems and the coincident diseases present. In this way, the students become familiar with the material in the hospital. The numerical results found by each student are added up and these figures used by them at home to make a composite graph of the complications (graph I).[3] Also, at home they study the assignment for the next day's work. This consists of the classification of tuberculosis and the modes of onset as given in Osler's *Textbook*. The following morning we make rounds in the ward, and each patient is questioned especially as to the onset in his case, bringing out the essential features; this shows the student the method of obtaining the information from the patient directly.

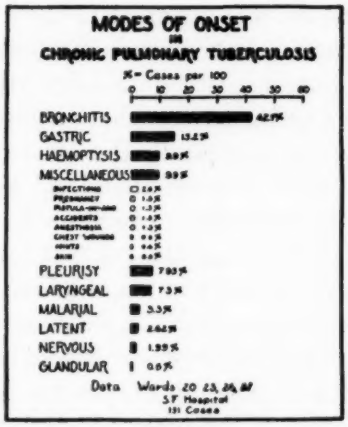
B: Modes of Onset: This subject is studied at home, and next day in the ward the students themselves question the patients in every detail as to the onset of the disease. If the onset was a haemorrhage, the student ascertains what the patient was doing at the time, the amount of blood lost, the number of haemorrhages, the treatment the patient had at the time, the subsequent care, etc.; for instance, recently a patient

[3] The graphs in this article were chosen from those done by the students as part of their work at home.

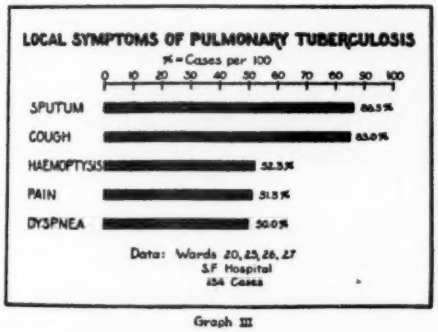
gave a history of having been perfectly well and attending commencement exercises for his A.B. degree. While singing the university hymn, his mouth filled with blood. The relating of this spectacular onset of the disease made a deep impression upon all the students in the section. In class, each student in turn tells about his patients with haemoptysis; thus, all the students have the benefit of the entire material in the wards.



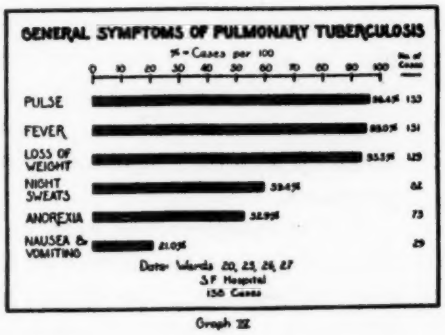
Graph I.



Graph II



Graph III



Graph IV

The various onsets are totalled in number and from these data the students make their graphs.

It is obvious that after students discuss with patients in their wards the various ways the disease is ushered in, they have a comprehensive viewpoint which cannot be obtained from books alone. We find that the students become tremendously interested in the patients and are most eager to relate the information obtained from them.

The work at home on this day consists of making a graph (no. II) showing in percentages the onsets from the data given in class. From this, the students readily see the relative frequency of the various onsets. At home they study, as well, the subject that is to be taken up with the patients the next day; that is, the local symptoms of the disease.

C: Local Symptoms: From questioning the patient, an account of the local symptoms of each patient is obtained. It was learned previously that "bronchitis" (graph II) was the most frequent onset of tuberculosis. Therefore, it is apparent to them that cough is the most constant local symptom. In the wards the students hear the patients cough and thus they learn to distinguish a dry hacking, or slight cough without sputum, from a productive cough, or a paroxysmal attack; at the same time they study macroscopically the sputum in the boxes at the bedside.

In class, the different local symptoms are discussed, the essential points of each being emphasized. The figures are again totalled and the data used to make the graph at home (graph III). Following this discussion, the salient features of miliary tuberculosis are reviewed from pathological-clinical and X-ray diagnosis.

D: General Symptoms: Similarly, the study at home prepares the students for taking up the general symptoms in the ward. For this work, the chart of the patient is necessary. With it, the student learns the type of temperature and the pulse-rate, sees the weight-curve; and from his questioning of the other general symptoms with the history before him, he acquires an excellent idea of the condition of the patient. In class, as with the local symptoms, each of the general symptoms is discussed: for example, fever. From the charts various types of fever are taken up with their significance in relation to the type of the disease and prognosis.

E: Examination: The examination of the chest now begins and the physical findings are charted on the diagram sheet (figure 1). The positive findings both in the history and in the clinical course are written on this sheet. The patients are assigned to the students in the order of the stage of the disease, first with, and then without complications, such as: incipient—one apex; two apices; moderately advanced—with or without cavities; advanced cases. The students work alone or in pairs, depending upon the type of case. In class, the student presents the patient he has studied. He gives the contact, onset, local and general symptoms, complications, laboratory findings, and physical signs, and reads the X-ray films. The entire group with the instructor examine the

T.B. BUR. P.4.

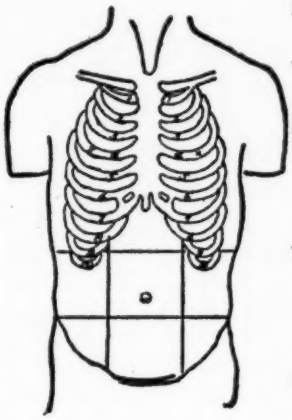
DEPARTMENT OF PUBLIC HEALTH
SAN FRANCISCO, CALIF.

.....

CHEST CLINIC
DIAGRAM SHEET

NAME OF PATIENT..... DATE..... 193.... CASE NO.....

INSP.
V. F.
BR. S.
V. S. { SP. V. / WH. V.
RALES



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V. S. { SP. V. / WH. V.
RALES

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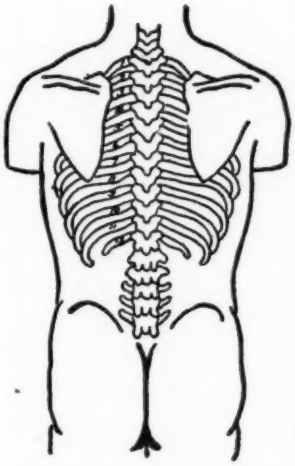
RALES



INSP.
V. F.
BR. S.
V. S. { SP. V. / WH. V.
RALES

On these sheets, the students chart the chest findings

In the space below the diagram, the salient points of the history are recorded.

Fig. 1

TABLE 1

Schedule of tuberculosis course, First semester: August 19–September 4, 1930

Section I

	Monday	Tuesday	Wednesday	Thursday	Friday
		August 19	August 20	August 21	August 22
9–11		Survey	Onsets—Ward	Local Symptoms (Wd.)	Gen. Symptoms (Wd.)
11–12		Clin. Conf.	Class	X-ray Conf.	
2–3		Survey		Local Symptoms (Wd.)	Class
3–4		Class		Class	Films { Incip. Mod. Adv. Adv.
	August 25	August 26	August 27	August 28	August 29
9–11	Rounds—Wd. 25	Study—Exam. Mod. Adv. Cases	Exam. Cases Adv. sans cavity	Rounds—Wd. 20	Rounds—Wd. 26—Adv. with cavity
11–12	Exam. Cases Incipient	Clin. Conf.	Class	X-ray Conf.	Exam. Cases
2–3	Class	Class		Fluoroscopy	Class
3–4	Exam. Cases Incipient	Exam. Cases Mod. Adv.		Class	Films
	Sept. 1	Sept. 2	Sept. 3	Sept. 4	
9–11	Rounds—Wd. 25	Rounds—Wd. 27	Exam. Cases	Class	
11–12	Exam. Cases	Clin. Conf.	Class	X-ray Conf.	
2–3	Class	Class		Practical Exam.	
3–4	Exam. Cases	Exam. Cases		Written Quiz	

A copy of this schedule is given to each student.

chest. A discussion follows, bringing out the essential features of the case and the probable prognosis.

F: X-ray: From now on begins the reading of the X-ray films for half an hour each morning. The subjects studied are in consecutive order according to the assignment for the day. As we have an extensive file of X-ray films of the chest, not only of all forms of tuberculosis, but also of all the diseases of the chest, the students see a vast amount of X-ray material. They are first taught to read the film of the normal chest. Following this, we take up tuberculosis, acute and chronic infections of the chest, and then the outstanding points in differentiation.

G: Complications: We study the complications according to systems. This is done at rounds and during the examination of patients. The rounds are made for an hour or more in different wards. Here the symptoms, complications, and clinical course of the disease are emphasized and the X-ray film of the patient is read at the bedside.

H: Fluoroscopy: For one hour the students study the fluoroscopic appearance of the chests of patients. First, a normal chest is demonstrated, to show the landmarks and respiratory movements in the anterior, posterior, and oblique positions. Then, in turn, are shown the patients with incipient, moderately advanced, and advanced tuberculosis. Following this, patients are shown who demonstrate thickened pleura, pleural effusion, pneumothorax, hydropneumothorax, large hilum glands, and other unusual or interesting chest conditions.

The students are allowed to examine as many patients as they possibly can, after having studied the three typical stages of the disease. At the end of the session, a practical and a written test are given. The graphs and diagram sheets are graded and returned to the students. (For schedule of class see table 1.)

TUBERCULOSIS COURSE[4]

Third Year

Section 1

First Day—Tuesday Aug. 19	A.M. Survey—Ward
	P.M. 2:00 Survey—Ward
	3:00 Survey—Laboratory
Second Day—Wednesday Aug. 20	A.M. Onsets—Ward
	11:00 Onsets—Class

[4] Sample of outline given to each student.

Third Day—Thursday Aug. 21	A.M. Local Symptoms—Ward P.M. Local Symptoms—Ward
Fourth Day—Friday Aug. 22	A.M. General Symptoms—Ward P.M. General Symptoms—Class
Fifth Day—Monday Aug. 25	A.M. Rounds Study—Examine incipient cases—ward P.M. Class—Examine patients Right apex Left apex Both apices Demonstration X-ray films, normal and incipient cases
Sixth Day—Tuesday Aug. 26	A.M. Study and examine moderately advanced cases P.M. Class Examine moderately advanced cases Films Pleural complications
Seventh Day—Wednesday Aug. 27	A.M. Study—Examine advanced tuberculosis with cavity 11:20 Class—Examine X-ray films Discussion: Spontaneous pneumothorax Hydropneumothorax Pyopneumothorax X-ray films
Eighth Day—Thursday Aug. 28	A.M. Rounds P.M. Class— Fluoroscopy (Patients demonstrated) Incipient Moderately advanced Advanced cases Fibrous pleurisy Effusion Pneumothorax Hydropneumothorax Examination of patients
Ninth Day—Friday Aug. 29	A.M. Rounds Examination of patients P.M. Class—advanced tuberculosis with pleurisy Examine fibroid tuberculosis X-ray films of Advanced with cavity Fibroid tuberculosis Intestinal cases
Tenth Day—Monday Sept. 1	A.M. Rounds—Ward Examination of patients P.M. Class—Discussion on Pneumonia Abscess Empyema X-ray films
Eleventh Day—Tuesday Sept. 2	A.M. Rounds—Ward Examination of patients X-ray films

	P.M.	Class—Discussion on Bronchitis Emphysema Bronchiectasis Asthma
Twelfth Day—Wednesday Sept. 3	A.M.	Examination of patients 11:00 Class—Review and discussion on Atelectasis Pneumokoniosis Neoplasm
Thirteenth Day—Thursday Sept. 4	A.M.	Class Review Practical examination Written quiz

(*Note:* This outline necessarily changes when there is an autopsy as staff and students attend.)

Assignments For Home Study[5]

1930	
1st Day Aug. 19	Onsets of chronic Pulmonary Tuberculosis
2nd Day Aug. 20	Local Symptoms
3rd Day Aug. 21	General Symptoms
4th Day Aug. 22	General classification of miliary tuberculosis General classification of pulmonary tuberculosis } 3 forms of each Review of physical signs of chronic ulcerative tuberculosis Review of physical signs of caseous pneumonia
5th Day Aug. 25	Respiratory complications Larynx Pleurae { Acute pleurisy / Fibrous pleurisy / Effusion
6th Day Aug. 26	Respiratory complications (Cont'd) Spontaneous pneumothorax Hydropneumothorax Pyopneumothorax
7th Day Aug. 27	Respiratory complications (Cont'd) Lungs Haemoptysis Compensatory emphysema Subacute disseminated Fibroid tuberculosis
8th Day Aug. 28	Gastrointestinal complications Mouth Intestines Ischio-rectal abscess Fistula-in-ano

[5] Each student receives a copy of this sheet with his respective scheduled dates.

9th Day	Nontuberculosis
Aug. 29	Pneumonia
	Abscess
	Empyema
10th Day	Bronchitis
Sept. 1	Emphysema
	Bronchiectasis
	Asthma
11th Day	Atelectasis
Sept. 2	Pneumokoniosis
	Neoplasm
12th Day	General Review
Sept. 3	
13th Day	Practical examination and written quiz
Sept. 4	

Second Semester

Treatments and special procedures

A: Medication: On the first day, the students are assigned to the various wards, and in the ward treatment room they study the special treatments. Each student is given a syllabus of the medical treatment of tuberculosis with the prescriptions used in the service. The students copy the list of the medication each patient is having and identify this with the medicines in the adjoining medicine cabinet as well as with the syllabus, thus becoming familiar with drugs and treatment. This serves as a sound apperceptive basis for the work with the patient later. Taking the chart to the bedside, the students question the patient about his condition and correlate symptoms and treatment.

From this they deduce for themselves the actual value of the general regimen in the care of a tuberculous patient as well as the efficacy of the various medications. At the regular rounds the following mornings, particular stress is laid upon treatment, the indication for special procedures, and discussions of the course of the disease with prognosis.

B: Tuberculin: This treatment is fully explained in class before it is demonstrated. The type of patient to which the tuberculin is given on this service is first described, the indications and counterindications for its use are discussed, and the methods for regulating the dose are explained. The students then see the tuberculin treatment given, and also study the temperature charts and the method of charting the dosage.

C: Rollier: The students see the patients taking the treatment (details given in lecture) and note the early erythema, the development of the pigmentation, and the resulting effects upon the skin. They receive a

copy of the directions together with the regulation forms used in recording the treatment.

D: Artificial Light Therapy: The effect of light treatment is studied on those patients for whom the lamp is considered to be more efficacious than the Rollier treatment, especially in skin, intestinal, and glandular tuberculosis.

E: Artificial Pneumothorax: One afternoon is devoted to instruction in artificial pneumothorax. The patients receiving the treatment are shown under the fluoroscope and the reasons for giving or not giving air on that particular occasion are seen. The students are taught to observe the amount of collapse, the presence of fluid in the pleural cavity, the shifting of the mediastinum, and the effect of adhesions. Then in the pneumothorax room they see the actual technique where each step is explained in detail. The new patients upon whom pneumothorax is to be started are brought up for discussion and the indications for treatment explained. They then see the first injection of air, followed by fluoroscopic examination.

F: Tuberculin Tests: A historical sketch is given of the various tests and these are studied in the wards on suitable patients and on those with questionable diagnosis. The intracutaneous test and its significance is studied in the Children's Ward.

G: Chest Surgery: There are always in the wards a number of patients who have had various forms of chest surgery performed, as well as many who are under observation for operation. A history of the course of the disease is given with the X-ray films and the indications for surgery and a discussion of the prognosis in each case. The actual performance of the operations is witnessed while the students are on the surgical service.

H: Tuberculosis in Children: One afternoon a week is spent in the Children's Ward. The majority of the patients are contacts; others are either suffering from glandular tuberculosis and actual infiltration of the lungs, or are being kept under observation until a definite diagnosis can be established. This affords ample material for explanations and discussions of childhood tuberculosis.

I: Laryngeal Tuberculosis: Laryngeal tuberculosis is studied one afternoon in the throat clinic, which is in charge of the resident laryngologist. The anatomical landmarks of the larynx are first reviewed. Following this, the various types of laryngeal lesions are demonstrated from our patients correlating the clinical symptoms and pathology. They see as well, during this session, the treatments given the patients.

J: Orthopedic Cases: One hour is devoted to observing the different forms of bone-and-joint tuberculosis under the supervision of the orthopedic consultant. The interpretation of these X-ray films is taught in conjunction with the clinical aspects of the case. There is an average of 15 orthopedic patients in the service.

K: Chest Clinic: The students spend two mornings in the chest clinic, which is connected with the hospital. First of all, they are instructed in the general plan of the tuberculosis work: the interpretation of the large wall-map of the city with its colored pins indicating the location of all active cases (those reported by private physicians and by the chest clinics of San Francisco); the mortality to date for the year; the function of the field-nurses in visiting and instructing the sick in the home; the method of bringing in all contacts for examination; and the follow-up work. Next comes the handling of old cases. The doctor in charge of the clinic questions the patient and so do the students. They learn from the questioning and from the clinical record the progress of the case. The sociological problem, an important factor in these patients, is correlated with the physical and mental condition. The students learn of the public agencies available for helping the family and the system of referring to other clinics when necessary. Each student works up at least one new patient, takes the history, examines him, orders an X-ray, does a tuberculin test when indicated, and prescribes the necessary medication. His work, of course, is checked by the instructor.

The time spent here is of great value. The students acquire a comprehensive view of tuberculosis from the public-health standpoint. Moreover, they learn that tuberculosis is not a hopeless disease or at best a lingering existence in a hospital bed. They see that treatment is more than palliative, that tuberculosis is compatible with normal living, and that patients actually get well. (For schedule of class see table 2.)

Assignments for Home Study[6]

1st day	January 14	Treatment of local symptoms
2nd day	January 15	Treatment of general symptoms
3rd day	January 16	Signs and symptoms of respiratory complications
4th day	January 17	Treatment of respiratory complications

[6] Since this paper was given, the schedule for the second semester has been changed; for this reason the sample schedule for 1935 is used.

TABLE 2

Schedule of tuberculosis course, Second semester: January 14–25, 1935

Section I

	Monday, Jan. 14	Tuesday, Jan. 15	Wednesday, Jan. 16	Thursday, Jan. 17	Friday, Jan. 18
9–10	Treatment Lists			Rounds—Wd. 26	Rounds—Wd. 23
10–11	Medication in Wards		Chest Clinic		Class
11–12		Clinical Conference		X-ray Conference	
2–3	Rounds—Wd. 19	Class		Rounds—Wd. 30 (Children's Ward)	Examine Cases
3–4	Ward Work	Ward Work			
	Monday, Jan. 21	Tuesday, Jan. 22	Wednesday, Jan. 23	Thursday, Jan. 24	Friday, Jan. 25
9–10	Rounds—Wd. 25		Tuberculin Treatment	Rounds—Wd. 26	Examine Cases
10–11	Examine Cases		Chest Clinic		Quiz
11–12		Clinical Conference		X-ray Conference	
2–3	Throat Clinic	Class		Rounds—Wd. 30 (Children's Ward)	Artificial Pneumothorax
3–4		Examine Cases			

* Since this paper was given, the schedule for the second semester has been changed; for this reason the sample schedule for the year 1935 is used.

5th day	January 18	Signs and symptoms of gastrointestinal complications
6th day	January 21	Treatment of gastrointestinal complications
7th day	January 22	Tuberculin treatment
8th day	January 23	Treatment of lymphatic complications
9th day	January 24	General review

C: Fourth Year

Each section of the class works for ten weeks in the general medical clinic of the Out-patient Department of the Medical School, and during this time any patient found to be suffering from symptoms suggestive of tuberculosis is seen in consultation with the chief of the Chest Clinic.

D: Interne Year

As internes, the fifth-year students at the San Francisco Hospital have two months in the Tuberculosis Service. The work is so planned that, in addition to their ward service, they assist in the special diagnostic and therapeutic procedures, that is,

(a) Artificial pneumothorax
(b) Throat clinic
(c) Chest clinic
(d) Genitourinary clinic
(e) Tuberculin treatment
(f) Orthopoedic clinic

During the spring semester, six of the internes who have already served on the Tuberculosis Service at the hospital, each have a month at the Sanatorium, thirty-three miles from San Francisco, an institution of 80 beds, where the early and convalescent patients are sent. In addition to the routine work, they have the opportunity of doing the fluoroscopy, taking and developing the X-ray films, and assisting with pneumothorax.

There is no doubt that instruction in the tuberculosis wards is of great value to the medical student. This is true, not only because of the vast amount of material available for learning the physical signs of the chest, but also because here alone can be studied the natural course of the disease in all its manifestations.

The students learn that tuberculosis is not hopeless; that rest, fresh air, and proper diet, the three things which will arrest a large number of cases of pulmonary tuberculosis, depend, according to Osler upon the physician's making the diagnosis early.

STUDIES ON THE BEHAVIOR OF TUBERCLE BACILLI WITHIN THE BODY

I. THE FATE OF VIRULENT AND AVIRULENT TUBERCLE BACILLI INJECTED INTRACUTANEOUSLY INTO NORMAL ANIMALS

H. J. CORPER[1] AND MAURICE L. COHN[1]

The problem of tuberculosis is complicated by numerous factors which within recent years have become more and more susceptible to accurate and controlled analysis. We now recognize certain pathogenic strains, the human, bovine, and avian tubercle bacilli, which can be biologically characterized by cultural and animal pathogenic qualities, as differentiated from a large group of undefined acid-fast bacilli, not true tubercle bacilli. In addition, certain avirulent tubercle bacilli must be recognized with no, or slight, pathogenic propensities for animals, characterized mainly by their history and reactions, and especially differentiated from the usual saprophytic acid-fast bacilli by growing restrictedly at approximate incubator temperature (37°C.). (1) (2) Furthermore, we are now fairly well conversant with animal susceptibility to the virulent strains as well as organic susceptibility in the animal in question. (3) More is known to-day regarding the routes of infection than in former years, but the fate of the bacilli is complicated by the problem of organic resistance, the lack of data on the effects of graded infection, as well as the resistance of the animal.

The difficulties encountered in following the bacilli are well illustrated by the fact that the nature of lysis *in vitro*, within the realm of natural laboratory control, is still an unsolved problem. (4) The fate of the bacilli can, however, be followed at present in the animal (or human) economy by means of suitably controlled animal or culture test methods. The use of the animal is restricted primarily to specific pathogenic strains, and thus control test must determine the range of quantitative efficiency for this test method. For example, the guinea pig cannot be used as a test for avian or avirulent bacilli, nor as efficiently for virulent human bacilli as for virulent bovine bacilli.

One criticism offered against the use of the animal is the presence of

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spontaneous tuberculosis and acute epidemic disorders. For exhaustive tests animal costs also become prohibitive. It is now recognized that the usual strain of tubercle bacilli can be recovered just as efficiently by culture as by animal inoculation (5), regardless of the route of injection. It is also well recognized that the factor of susceptibility of some animal species to virulent tubercle bacilli may be changed by previous contact with tubercle bacilli, so that any study on animals must give due consideration to this point. In an earlier study from this laboratory, it was found, in normal rabbits and dogs (6) given intravenous graded nonlethal amounts of virulent human tubercle bacilli, that these bacilli are actively destroyed (gauged by guinea-pig inoculation) in the various organs at a variable rate. Although these experiments dealt with definite amounts of bacilli given intravenously, their organic deposition left exact evaluation in doubt.

To learn more regarding the nature of tuberculosis, the actual fate of the bacilli from a definite site of inoculation appeared worthy of exact study. The problem of multiplication, dissemination, and death in a focus with knowledge regarding the pathogenicity of the strain merited consideration. For this purpose, the skin possessed definite experimental advantages, and especially since certain valuable pioneer studies in the guinea pig were available as a guide. In 1920, Krause (7) presented his important contributions on immunity to tuberculosis in guinea pigs, although he apparently infected with large amounts (not accurately determined) of virulent human tubercle bacilli (H37). He found that the skin lesion developed in a definite chronological sequence, differing from that in allergic (immune) guinea pigs. In 1924, Krause (8) showed that there occurred a rapid dissemination of bacilli from the site of injection of a heavy suspension of virulent human tubercle bacilli in normal guinea pigs. In 1925 (9) he pointed out that

> (1) in normal nonimmune guinea pigs, tubercle bacilli inoculated intracutaneously or subcutaneously are carried almost immediately (within an hour) from the portal of entry by the lymphatics; (2) within three or four days they have made the circuit of the body; (3) in immune (allergic) animals, their transmission is greatly retarded; (4) they remain fixed at or near the portal of entry for about seven days; (5) they do not reach the regional lymph nodes until two weeks after injection; and (6) they do not become generalized throughout the body until three to four weeks after infection.

Willis (10) working with Krause on the foregoing study, reported similar results when using inexact heavy suspensions of human tubercle

bacilli in guinea pigs. In order to confirm his inoculations, he injected "impalpable prussian blue" intracutaneously into guinea pigs. Upon examination, it was found that in about half of the animals lymphatic injection of greater or less degree occurred. One capillary filled to 20 mm. and a few 5 to 15 mm. He says, "It was therefore likely that of the animals which showed very early dissemination (one and two hours) some may have represented lymphatic injection."

The fact that the quantitative concept must be applied to any problem of the injection of a particulate suspension like tubercle bacilli in fine division suggested that we repeat these injections with varying amounts and concentrations of an easily discernible substance entirely insoluble yet finely divided. India ink, on the basis of previous experience (11), seemed to be best suited for this purpose.

For the purpose of testing the fate of intracutaneously injected particulate material, guinea pigs were given intracutaneous injections of varying amounts of pure india ink (Higgin's special white label in which the particles are even finer than tubercle bacilli) in varying dilutions. At certain intervals thereafter, test guinea pigs were examined both externally and internally. The skin was carefully examined when fresh and also after preservation. In one series of guinea pigs, a constant volume of solution was injected intracutaneously (either 0.2 or 0.1 cc.), using a tuberculin syringe accurately calibrated and different concentrations of india ink; while in another series the volume ranged from 0.2 cc. to 0.05 cc., and the concentration of india ink was constant (either 20 per cent or 5 per cent of full-strength white label Higgins). The results indicated that the primary and important determinant of the area of deposition of the india ink was the concentration. The intracutaneous injection of 0.2 cc. of 20, 5, 1, and 0.1 per cent india ink, for illustration, produced a deposition proportional to the concentration (measuring from the internal skin side) 14, 8, 5, and 3 mm. in diameter respectively. There were noted two other factors which assume practical importance in the findings. One was that the injection of the higher concentration may result in definite lymphatic extension, in one case being traceable for about 4 to 5 cm. from the intracutaneous site to several small lymphoid areas; the second was that there occurred evidence of a definite filtration concentration of the particulate material at the needle point of inoculation. (See figure 1.) Of secondary importance was the volume of the injection. When 0.2, 0.1, and 0.05 cc. of 5 per cent india-ink solution was injected, no striking difference in the deposition was noticed exter-

nally, except that the depth of color was denser for the larger volume while the extent as measured on the internal skin surface ranged for these amounts as follows: 8, 6, and 4 mm. in diameter, respectively.

Applied to the intracutaneous injection of tubercle bacilli, it would appear reasonable to expect that heavy concentrations would tend to seek lymphatic outlet and thus be disseminated, while smaller amounts would, in the majority of cases, tend to be filtered and concentrated at the site

PLATE 1

Fig. 1. The zone of spread of india ink following intracutaneous injection in 0.2-cc. amount (viewed from the inside). Note the lymphatic spread (marked by arrows) following the injection of a high concentration of particulate material (0.2 cc. of 20 per cent india ink). Note also striking gross similarity between 1 per cent and 5 per cent concentrations and the filtration deposition of the 0.1 per cent concentration of india ink. (In the picture the blood-vessels also photograph black, and must not be confused as containing india ink).

Fig. 2. The effect of india ink (5 per cent) on the development of the skin lesion sixteen days after the intracutaneous injection of graded amounts of fine suspensions of virulent bovine tubercle bacilli in the guinea pig. The "R" (right) lesions were produced by india ink and tubercle bacilli, and the "L" (left) by the tubercle bacilli alone. The arrow points to the site of injection of india ink (5 per cent) without bacilli.

Fig. 3. The skin lesions two months after the intracutaneous injection of graded fine suspensions of virulent human tubercle bacilli H7 (containing 5 per cent india ink). Note ulceration at this time of the lesion down to the 10^{-6} dilution.

Fig. 4. The same as fig. 3 taken at the six months' interval. Note more extensive ulceration and absence of tendency to heal.

Fig. 5. The skin lesions two months after the intracutaneous injection of graded suspensions of virulent bovine tubercle bacilli (Bovine virulent) (containing 5 per cent india ink). Note extensive ulceration of the lesion to the 10^{-8} dilution at this time interval. The virulent bovine strain is more pathogenic for guinea pigs than the virulent human strain.

Fig. 6. The skin lesions two months after the intracutaneous injection of graded suspensions of virulent avian tubercle bacilli (Avian 3) (containing 5 per cent india ink). Note ulceration of the lesion in the 10^{-2} dilution at this time. Later (after four months) all these lesions were healed.

Fig. 7. The skin lesions two months after the intracutaneous injection of graded suspensions of avirulent human tubercle bacilli (Human avirulent) (containing 5 per cent india ink)—a strain revealing no pathogenicity for any of the animal species used in these tests. Note slight lesion in one of the sites in the 10^{-2} dilution at this time. There was no evidence of progression and later the small lesion gradually resolved.

of inoculation, especially since the particles of the india ink used were finer than fine suspensions of tubercle bacilli. Pertinent to this comparison is the fact previously recorded by us (12) that, on the basis of volumetric comparison of Higgins's white label india ink with fine suspensions of tubercle bacilli, about 0.2 cc. of carbon sediment is present in 1 cc. of ink; and 100 mgm. of tubercle bacilli gives a like sediment of about 0.2 cc. measured in a graduated centrifuge-tube after centrifugation

FIGURE 1.

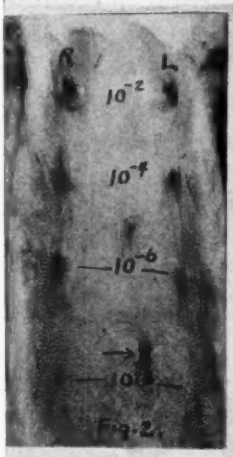

Fig. 2.

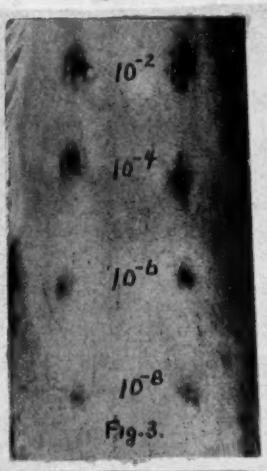

Fig. 3.

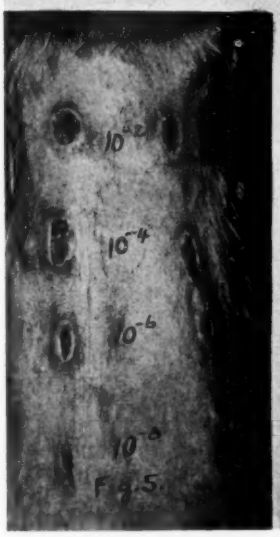

Fig. 5.

Fig. 6.

Fig. 7.

PLATE 1

at about 3000 *r.p.m.* Although this can only be a crude measure, it suggests that, in experiments using even as much as one milligram of tubercle bacilli, this amount is less than that giving lymphatic spread with india ink. Another fact noted was that with the inert particulate material, india ink, the area of spread remained unchanged macroscopically regardless of the time after injection even to about a year. This also served as a permanent tatoo mark of the site of inoculation and was used in the experiments to follow.

PLATE 2

Fig. 8. Skin lesions in the dog two weeks after the intracutaneous injection of graded amounts of fine suspensions of virulent human tubercle bacilli (H7) (in 5 per cent india ink). Note ulceration of 10^{-2} concentration site at this time. This occurs similarly in the rabbit with H7.

Fig. 9. Skin lesions in a dog four months after receiving the same intracutaneous injections of H7 as fig. 8. Note the healed scars at this time resulting from ulceration of lesions 10^{-2} and 10^{-4}.

Fig. 10. Skin lesions in the dog one month after the intracutaneous injection of graded amounts of fine suspensions of virulent bovine tubercle bacilli (Bovine virulent) in 5 per cent india ink. Note ulceration at this time in 10^{-2}, 10^{-4}, and in one of 10^{-6} concentrations.

Fig. 11. Skin lesions in a dog four months after receiving the same intracutaneous injections of Bovine virulent as fig. 10. Note the healed scars at this time resulting from ulceration of lesions 10^{-2}, 10^{-4}, and 10^{-6} but not in 10^{-8}. Healing with scar-formation is the usual termination of such lesions in the dog.

Fig. 12. Skin lesions in the rabbit two months after the intracutaneous injection of graded amounts of fine suspensions of virulent bovine tubercle bacilli (Bovine virulent) in 5 per cent india ink. Note ulceration at this time in 10^{-2}, 10^{-4}, and 10^{-6} but not in 10^{-8} concentrations.

Fig. 13. Skin lesions in the rabbit two months after the intracutaneous injection of graded amounts of fine suspensions of virulent avian tubercle bacilli (Avian 3) in 5 per cent india ink. Note ulceration at this time in 10^{-2}, 10^{-4}, and one of the 10^{-6} concentrations. After six months all these lesions were healed.

Fig. 14. Skin lesions in the dog four months after the intracutaneous injection of graded amounts of avirulent human tubercle bacilli (Human avirulent). Note healed scar in one of the 10^{-2} lesions. Identical findings were obtained in the rabbit. In no case was there any evidence of progression of the lesions with this tubercle bacillus.

PREPARATION OF SUSPENSION OF TUBERCLE BACILLI

Any method of preparing quantitative suspensions of tubercle bacilli which resorts to filtration or centrifugation to remove lumps introduces a marked inaccuracy as to the exact amounts of bacilli in the final suspensions obtained. It was for this reason that the study of the preparation of suitable quantitative suspensions was pursued many years ago which culminated in the technique described earlier (13). Many substances and chemicals were tried for the purpose of assisting the dispersion of

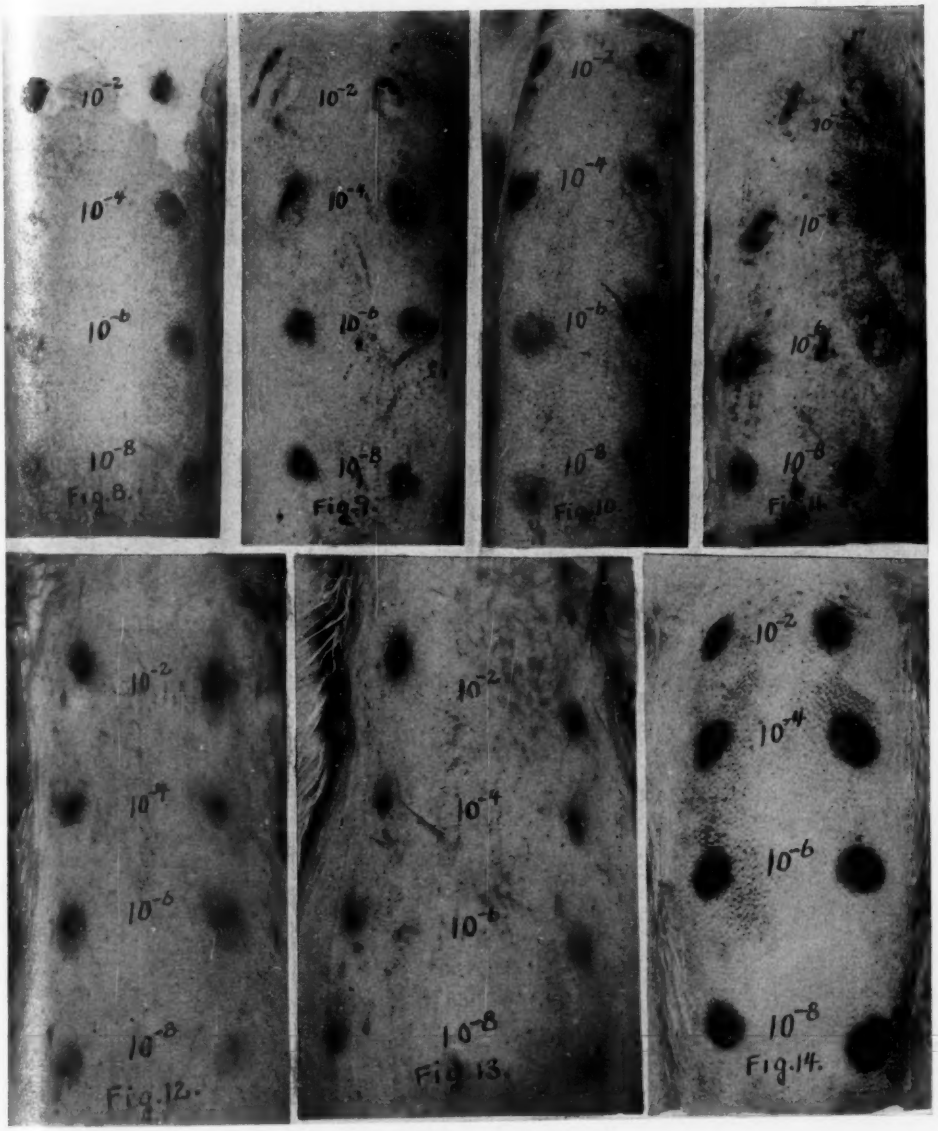

PLATE 2

the bacilli from small amounts of accurately weighed bacillary masses to fine equally divided suspensions, but many of these substances, such as soaps, saponin, gelatine, etc., proved either toxic or unsatisfactory for the purpose. However, sodium taurocholate (Eastman Kodak Co.) because of its surface-tension lowering propensities and lack of toxicity in suitable concentration proved a valuable aid in dispersing the bacilli. In an earlier paper (14) it was found that even a one per cent solution of sodium taurocholate incorporated in egg and potato media had no appreciable inhibitory effect.

Subsequent tests *in vitro*, with 0.5 per cent sodium taurocholate ground with the bacilli and also used as a diluent which remained in contact for several hours, had no appreciable effect on small numbers of bacilli, as was determined by the growth of dilutions containing as little as 0.000,000,01 mgm. bacilli per cubic centimetre. There was no effect of this treatment with 0.5 per cent sodium taurocholate on the virulence of the bacilli as tested in guinea pigs. Therefore, sodium taurocholate has been used to prepare satisfactorily fine suspensions of tubercle bacilli.

The method of preparing a suspension consists of weighing accurately about 10 mgm. moist young culture (about 3 to 5 weeks old) in a sterile graduated 15-cc. centrifuge tube. The bacilli are carefully ground on the glass tube by means of a sterile rounded glass rod slightly smaller than the bowl of the tube. Then one drop of sterile 0.5 per cent sodium taurocholate is added and the bacilli are thoroughly ground in this with a continual rotary motion. Finally, four or five drops more of the taurocholate solution are added intermittently a drop at a time after each grinding. After the grinding is completed, requiring usually five to ten minutes, 0.9 per cent sterile salt solution is added first by drops and mixed carefully with the bacilli and finally enough to give the desired concentration of the suspension. The final suspension from which further dilutions are to be made must possess a uniform milky opalescence without lumps or particles and contain 1 mgm. of bacilli per cubic centimetre. The dilutions are then made with sterile saline solution in stages of one to ten. In order to avoid contamination while manipulating the dilutions, it is well to have a separate small flask of saline solution for each dilution to be prepared. It is also advisable to use individual 1-cc. sterile pipettes for each transfer, so that one cubic centimetre is transferred to a 15-cc. centrifuge tube and to this is added the 9 cc. of sterile saline solution.

THE EFFECT OF INDIA INK ON THE TUBERCLE BACILLI

In earlier studies (12) it was found that a good nutrient medium (inspissated glycerol egg-yolk) containing 5 per cent Higgins's best-grade white label india ink exerted no detrimental influence on the growth of small plantings of virulent human tubercle bacilli. Likewise, when the suspensions of various strains of tubercle bacilli (human, bovine, and avian) contained 5 per cent india ink, and these suspensions were planted on inspissated glycerol-egg-yolk medium, no inhibition of the growth of small numbers (0.000,000,01 mgm.) of these tubercle bacilli could be noted.

In order to determine the effect of the india ink (as a marker) upon the development of the local tuberculosis, guinea pigs were given intracutaneous injections of varying concentrations (0.2 cc. of 0.01, 0.000,1, 0.000,001, and 0.000,000,01 mgm. per cubic centimetre) of fine suspensions of virulent human or bovine tubercle bacilli. The suspensions injected on the left side were identical to those injected on the right side except that they contained 5 per cent india ink. As controls, the same concentration and amount of india ink were injected intracutaneously between the sites of the injection of the suspensions of tubercle bacilli. Although the india ink itself produced a slight transient induration in this concentration, it had no effect upon the resulting lesions produced by either the virulent human or bovine tubercle bacilli, while it served as an efficient marker for locating the site of injection in the absence of development of a lesion. Where lesions developed, their course was identical to that in which india ink was present. (See figure 2.)

FATE OF TUBERCLE BACILLI AFTER INTRACUTANEOUS INJECTION

In order to determine the fate of the various strains of tubercle bacilli injected intracutaneously in varying amounts in the different animal species, a series of animals were given duplicate injections (0.2 cc. of 0.01, 0.000,1, 0.000,001, and 0.000,000,01 mgm. per cubic centimetre) of graded amounts of fine suspensions of the tubercle bacilli in question. Five per cent india ink was used to mark the site of inoculation so if no discriminating lesion developed, the site could be appropriately located without difficulty. In each case at least duplicate animals were used for each test to ensure the results as representing the true findings. The strains of tubercle bacilli used were a recently isolated virulent strain of human tubercle bacilli (H7), an avirulent strain of human tubercle bacilli (Human avirulent) (15), a virulent strain of

bovine tubercle bacilli (Bovine virulent, a glycerophilic laboratory strain), and a virulent avian strain (Avian 3), isolated several years ago from the liver of a pigeon. The animals used were the guinea pig, the rabbit, the rat, and the dog. The tests were carried for from six months to one year from the time of inoculation and consistent with the life-span of the experimental animals.

METHOD USED FOR RECOVERING THE TUBERCLE BACILLI

In all cases, the previously described 6 per cent sulphuric-acid method followed by 2 per cent sodium-bicarbonate neutralization (16) before planting was used to recover the tubercle bacilli. The tissues after washing with sterile saline (0.9 per cent sodium chloride) were planted on four tubes each of crystal-violet potato-cylinder medium (17) (18) and four tubes of inspissated egg-yolk medium (11).

In order to obtain clean tissues, especially from dirty laboratory animals, fairly free from extraneous contaminations which might interfere with obtaining satisfactory culture results for recovery of tubercle bacilli, all aseptic precautions were used to obviate contamination from this source. The animals were carefully shaved, the skin was washed with soap and water, and the soap was thoroughly removed by careful washing with water. Then it was further cleansed by at least three applications of an equal volume of pure acetone and 95 per cent ethyl alcohol, allowing the acetone-alcohol mixture to evaporate completely between each application. The animal was then rapidly electrocuted and each site of injection was removed, using a separate sterile forceps and scissors for each site. The tissue was then thoroughly and finely cut up in a sterile chemical porcelain crucible, using the scissor point for this purpose. The methods of using mechanical grinders, mortar and pestle, etc., all failed of the purpose of obtaining fine devision with the skin as well as the lung. Soft tissues like the liver and spleen were ground in a sterile mortar using a pestle for this purpose. In each animal, eight specimens of skin (four in duplicate) and specimens from the lung, liver, spleen, and kidney were submitted to culture test, each being cultured on 8 tubes of medium (four of potato and four of egg-yolk).

The results of the culture findings were assembled into tables (eliminated for lack of space) given under the division of the animal used for test purposes and including in each table the results for the various strains of tubercle bacilli—human, bovine and avian. In evaluating the results recorded in the tables, it must be remembered that this must be done in the

light of the method used for test (19). The culture method (and this is true also for the guinea-pig test with virulent strains for this animal) using good nutrients, such as potato and egg, and properly prepared without the presence of inhibitory substances (antiseptics, dyes, etc.) will readily disclose very small numbers of bacilli to a dilution of 0.000,000,01 mgm. per cubic centimetre. In our hands, exact enumeration of colonies has never proved feasible, although approximate concentrations of bacilli can be determined at times by the rate of appearance of growth and the nature of this growth (20). In these experiments, it must also be remembered that the presence of the bacilli in the organs has resulted from 8 intracutaneous injections with a total of only about 0.004 mgm. tubercle bacilli.

An examination of the data recorded in our tables revealed that, in the guinea pig, following the intracutaneous injections in a number of sites of a total of about 0.004 mgm. *avirulent human* tubercle bacilli (apathogenic for the guinea pig), there was no evidence of multiplication of these bacilli either at the skin site of inoculation or in the organs. There was no dissemination following these injections from the skin to the internal organs, except in one isolated instance among many tests when it was recovered with difficulty from the spleen. The bacilli were not recoverable from the skin sites after six to nine months after inoculation.

The data with *virulent human* and *bovine* tubercle bacilli (pathogenic for guinea pigs) injected intracutaneously in amounts as stated above revealed evidences of multiplication of the bacilli at all the intracutaneous sites of inoculation. Dissemination into the internal organs occurred within one to two weeks, with multiplication of the bacilli there. Finally, generalized tuberculosis resulted fatally in about two to four months with the bovine bacilli and after nine months with the human bacilli.

The data with the *virulent avian* tubercle bacilli (apathogenic for guinea pigs) revealed no evidences of multiplication at the intracutaneous sites of inoculation. There was, however, evident dissemination to the internal organs within one week, but no evidence of multiplication there or of the development of tuberculous lesions. The bacilli in the organs were not recoverable after two months, and they could not be recovered from the site of inoculation in the skin after six to nine months.

The data recorded in a table on the *rats*, animals that are practically insusceptible to any of these strains of tubercle bacilli given in moderate doses, showed a persistence of all of these strains at the site of inoculation up to six months but also definite evidence of a decrease in the number

of viable bacilli in the sites. The occasional dissemination to the internal organs was again evident, being more conspicuous for the reputed pathogenic strain (bovine) for this animal. In no case were gross tuberculous lesions found in the rat following the intracutaneous injections given.

An examination of the data recorded in another table indicated that in the *rabbit*, following the intracutaneous injection in a number of sites of a total of about 0.004 mgm. of *avirulent human* tubercle bacilli (apathogenic for the rabbit), no multiplication occurred at the sites of inoculation; and the evidence pointed to their being absent, in viable form, from the site of inoculation after six months. In no case were the bacilli recovered from the internal organs.

The results with the *virulent human* tubercle bacilli (apathogenic for the *rabbit*) are similar to those obtained with the avirulent *human* tubercle bacilli, except that in one isolated case the bacilli were recovered from the spleen of one animal at two weeks after injection.

The results with the *virulent bovine* tubercle bacilli (moderately pathogenic for the rabbit) (5) showed both evidences of slight multiplication at the site of inoculation, as well as more frequent dissemination into the internal organs than with avirulent bacilli. Although the amount injected is not capable of producing an early generalized disease, intercurrent conditions among this series made it difficult to carry the tests for longer intervals in these experiments.

The results with the *virulent avian* tubercle bacilli (moderately pathogenic for the rabbit) (5) recorded in this table indicated no appearance of multiplication of these bacilli at the site of inoculation, and there occurred only rare dissemination to the internal organs (in one animal the bacilli were recovered from the spleen after two weeks). The findings also indicate a disappearance of the bacilli from the site of inoculation in viable form after four to six months.

An examination of the data recorded in a fourth table revealed that in the dog, after the intracutaneous injection in a number of sites of a total of 0.004 mgm., there is a definite tendency for the bacilli to disappear within six months from the sites of inoculation, in viable form. The amount injected intracutaneously was not pathogenic for the dog (5) with the strains used in these tests. However, *virulent human* and *bovine* bacilli, in amounts approximating one milligram, could produce a lethal acute generalized disease. The tendency for dissemination of the bacilli to the organs was slight.

DISCUSSION

Any interpretation of tuberculosis must start with facts based on scientific observations applied within the practical sphere of data accumulated and built into a conception measuring up to actual occurrences in nature. It is obvious that naturally no individual becomes infected spontaneously with tens or hundreds of milligrams of bacilli, except possibly in the rare case of a laboratory or slaughter-house inoculation with a coincident perforating wound. It is, however, likely and of common occurrence that a small number of tubercle bacilli enter the human or animal economy under various and varying conditions. Pertinent to the outcome, there enters the problem of the strain, the numbers of bacilli, and the nature of the host and its reactions. It was for the purpose of elucidating these points that the foregoing investigations were pursued: first, in several species of normal animals with the various strains and virulences of the bacilli; and, finally, in "so-called" immune animals, although the nature of immunity is still being actively discussed and will be taken up by us in a later paper. In the past, anatomical tubercles have been used as the index of infection in tuberculosis because of lack of better criteria; but since anatomical tubercles are dependent on innumerable influencing factors, an interpretation in tuberculosis with such a guide could give no insight into the actual number and conditions of the assaulting invader—the tubercle bacilli. Then, to complicate the situation, at least from an experimental standpoint, virulence has been a term only crudely defined and never submitted to actual quantitative definitition. Until this has been satisfactorily consummated, disagreements regarding virulence will arise; and, for tubercle bacilli, a quantitative definition becomes particularly pertinent because even dead bacilli can produce tubercle,—the apparent past criterion of pathogenicity. Since tubercles may fluctuate, and since tubercle bacilli are the acknowledged criterion upon which pathogenicity must be built, as much knowledge as can be obtained regarding their fate in the animal body is pertinent to making a satisfactory definition of virulence. It was for this reason that the fate of four different strains of tubercle bacilli (Human virulent and avirulent, Bovine virulent, and Avian virulent) was studied in four different animal species. About a decade ago, Krause had already given valuable data on the guinea pig injected with virulent human tubercle bacilli, but it required more exhaustive elaboration even for this animal, since he had available at that time no accurate criterion for studying the fate of avirulent tubercle bacilli.

With the acquisition of methods adaptable to both avirulent and virulent tubercle bacilli, including delicate culture and animal tests, with knowledge regarding organic susceptibility in various animal species as well as man (21), it became possible to pursue more elaborate studies on the fate of the different strains of tubercle bacilli in the body. The results of these studies in normal, nontuberculous animals injected intracutaneously are included in the study reported here following another study in which the blood was examined in a series of animals after intravenous injection. From these studies, it was concluded that the bacilli are rapidly removed from the blood except when present in overwhelming amounts and producing an acute generalized disease with early death of the animal, and indicates that tuberculosis is not a bacillaemia but rather is complicated by an occasional embolic spread (5).

The results of the intracutaneous studies indicate that, when there is no metastatic tuberculosis resulting from such inoculation of small amounts of tubercle bacilli (0.004 mgm.), the bacilli in these sites or the bacilli which had occasionally disseminated from the site of inoculation to the internal organs, show a definite tendency to disappear in viable form. The strictly avirulent (apathogenic) human strain showed less tendency to leave the site of inoculation than the other strains studied.

In earlier studies it was shown that even avirulent bacilli for a certain species of animal (dog) may remain viable for a long time under forced experimental conditions when injected in large amounts (hundreds of of milligrams intravenously), but the bacilli are apathogenic and do not produce evidences of multiplication or of a progressive disease in this animal (14), nor do they lose their original apathogenic properties for other species (guinea pig) during this period of residence in the dog. Likewise, a young dog recovering from an almost lethal injection of virulent human tubercle bacilli resulting in permanent secondary bony structure changes, revealed no microscopic organic tuberculous changes after twenty-one months, nor could viable tubercle bacilli be found in any of the organs by guinea-pig inoculation test (6). In many cases, to discuss tuberculosis without detailed quantitative conceptions and knowledge regarding virulence and animal (general and organic) susceptibility might lead to obviously overdrawn impractical deductions.

SUMMARY

1. The factors determining the zone of spread of particulate material relative to the spread of tubercle bacilli in intracutaneous sites of injection in animals is determined.

2. An improved method of preparing a reproducible satisfactorily uniform fine suspension of tubercle bacilli, capable of being diluted to an extinction point of 0.000,000,01 mgm. of tubercle bacilli per cubic centimetre, is described.

3. India ink (pure suspension) in 5 per cent concentration has no effect on the growth of tubercle bacilli *in vitro* or on the development of the lesion resulting from the intracutaneous injection of tubercle bacilli and can be used to mark the site of inoculation of the tubercle bacilli.

4. Tubercle bacilli, avirulent or apathogenic for an animal species, when injected intracutaneously in small amounts, have a definite tendency to disappear in viable form from the site of inoculation in that species. There is also a tendency for early disappearance from the occasional site of deposition in the internal organs.

5. Universally avirulent human tubercle bacilli (apathogenic to all animal species) also disappear in viable form from the intracutaneous site of inoculation and possess less tendency to disseminate to internal organs.

6. Virulent tubercle bacilli for the species in question tend to multiply at the site of inoculation, as well as the site of dissemination to the organs, unless the amount inoculated is too small to produce ultimate tuberculous changes, when they also tend to disappear in viable form from the site of intracutaneous inoculation and of dissemination. There is a definite quantitative relation between pathogenicity and virulence in the susceptible species to a specific virulent strain.

Grateful acknowledgment is due Mr. L. D. Miller for his coöperation in assisting in the performance of many of the tests required to obtain the data recorded in this study.

REFERENCES

For References see end of paper V, this series, where all references are assembled for the series.

STUDIES ON THE BEHAVIOR OF TUBERCLE BACILLI WITHIN THE BODY

II. THE FATE OF VIRULENT AND AVIRULENT TUBERCLE BACILLI INJECTED INTRACUTANEOUSLY INTO IMMUNE PREVIOUSLY INOCULATED GUINEA PIGS

H. J. CORPER,[1] MAURICE L. COHN[1] AND A. P. DAMEROW[1]

At the present time, with experimental evidence available which dates from the recognition of the changed reaction to tubercle bacilli in the animal economy by Koch, it can hardly be doubted that there is a protection afforded the tuberculous animal by the first infection. Paradoxes, however, arise on attempting to theorize on facts and to build elaborate unproved concepts which aim to frame all the minute details into a complete all-encompassing picture of a complicated chronic disease without due consideration for the obvious facts in the phase of the case under scrutiny, and which are experimentally reproduced to resemble natural conditions. Koch required virulent tubercle bacilli and tubercle for the establishment of immunity because his knowledge was limited regarding the bacilli.

Since then (22) (23), this conception has guided many students of immunity in tuberculosis. Calmette extended attention to avirulent tubercle bacilli and immunity; and still there were individuals who would only be content with the demonstration of absolute immunity, probably because they could not conceive of the existence of relative values in this disease. Yet, methods for exact relative evaluation were not available prior to a decade or so ago. Suspensions of tubercle bacilli were considered the same, regardless of amounts, if they would infect the animal in question. Notwithstanding their immunity, even the highly susceptible guinea pigs were tested with overwhelming doses of virulent tubercle bacilli, which, no doubt, would still lead to generalized disease within reasonable time with one-millionth or less of the amount used,—a striking range of difference. In spite of this, immunity was still evident and important information accrued from these studies, if the data were properly selected on the basis of present criteria. Thus,

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important studies by Krause and his co-workers concern us particularly here because they used intracutaneous infections which could be repeatedly observed in the same animal over an extended period of time. Clearly, this was the only method available for exact and controlled repeated observations. As early as 1920, Krause and Peters (7), recognizing the difficulty that beset them in duplicating suspensions of tubercle bacilli, found that there occurred

(1) an immediate reaction on the part of the tuberculous animals which is absent in the normals; (2) an early development of nodule in the former which is again lacking or less well defined in the controls (one week); (3) a tendency of the lesions in the tuberculous guinea pig soon to come to a standstill, while at the same time (2 weeks) those in the controls are now rapidly developing; and (4) a later tendency of lesions in the tuberculous animal to retrogress and heal, while in the controls to persist and progress.

In 1924, Krause, (8) recorded a difference in rate of dissemination of the bacilli from an intracutaneous site in normal and allergic guinea pigs, and the following year Willis (24), reporting in detail on these facts, noted.

In marked contrast to the rapid dissemination of tubercle bacilli from a point of inoculation in the skin of normal guinea pigs of first infection, dissemination of bacilli is materially retarded in the skin of guinea pigs of reinfection, made allergic or immune by previous infection with tubercle bacilli.

In elaboration of findings on normal animals following the fate of various strains of tubercle bacilli intracutaneously injected into guinea pigs, rats, rabbits, and dogs, and as a necessary requisite for experiments being pursued on immunity in actual infection experiments, it became essential to determine the fate of the bacilli in immune and previously injected or infected animals. The studies with normal animals showed that tubercle bacilli, avirulent or apathogenic for an animal species, when injected intracutaneously in small amounts, have a definite tendency to disappear in viable form from the site of inoculation in that species. There is also a tendency for early disappearance from the occasional site of deposition in the internal organs. Universally avirulent human tubercle bacilli, apathogenic to all animal species, also disappear in viable form from the intracutaneous site of inoculation, and possess less tendency to disseminate to internal organs. Virulent tubercle bacilli for the species in question tend to multiply at the site of inoculation as

well as the site of dissemination to the organs unless the amount inoculated is too small to produce ultimate tuberculous changes; then they also tend to disappear in viable form from the site of intracutaneous inoculation and organic deposition.

The methods used in this study are essentially the same as those recorded in an earlier paper (Paper I, this series). When avirulent tubercle bacilli are given intravenously and the fate of the intracutaneously injected virulent bacilli are being followed, it becomes evident that the culture method is unsuited to discriminate between the virulent and the avirulent bacilli in the organs. Since, with a highly virulent strain of human or bovine tubercle bacilli, the guinea pig is as delicate as the culture method for determining the presence of these bacilli, this animal could be adequately used for differential purposes.

THE FATE OF VIRULENT HUMAN TUBERCLE BACILLI INTRACUTANEOUSLY INJECTED INTO IMMUNE GUINEA PIGS

For the purpose of immunizing the guinea pigs, they were given intravenous injections (ear-vein) of one milligram in one cubic centimetre of fine suspension of avirulent human tubercle bacilli. This injection produced in normal guinea pigs only a transient acute splenomegaly, which left no tubercles and completely resolved after two months. This injection affords an almost complete immunity against infections a hundred to a thousand times the minimum infecting dose with virulent human and bovine tubercle bacilli in the guinea pig, and shows a definite retardation of infections with larger doses. (The data on this will be reported in a subsequent paper).

An examination of the results recorded in the first table revealed little difference from the culture findings with normal guinea pigs (Paper I, this series). However, in evaluating the results it must be remembered that both the culture and guinea-pig test reveal the presence of small numbers of bacilli even though there exist no evidences of macroscopic or microscopic tuberculosis. Macroscopically, there is a striking difference in the amount of tuberculous involvement between the immune and normal guinea pigs which will be presented in greater detail in a subsequent paper. None of the animals recorded here showed macroscopic organic tuberculosis until the six-months interval in spite of the fact that relatively large intracutaneous injections with virulent human tubercle bacilli (H7) were given. As far as comparisons are possible, it would appear that the number of bacilli in the organs are far more numerous in the normal than in the immune animals.

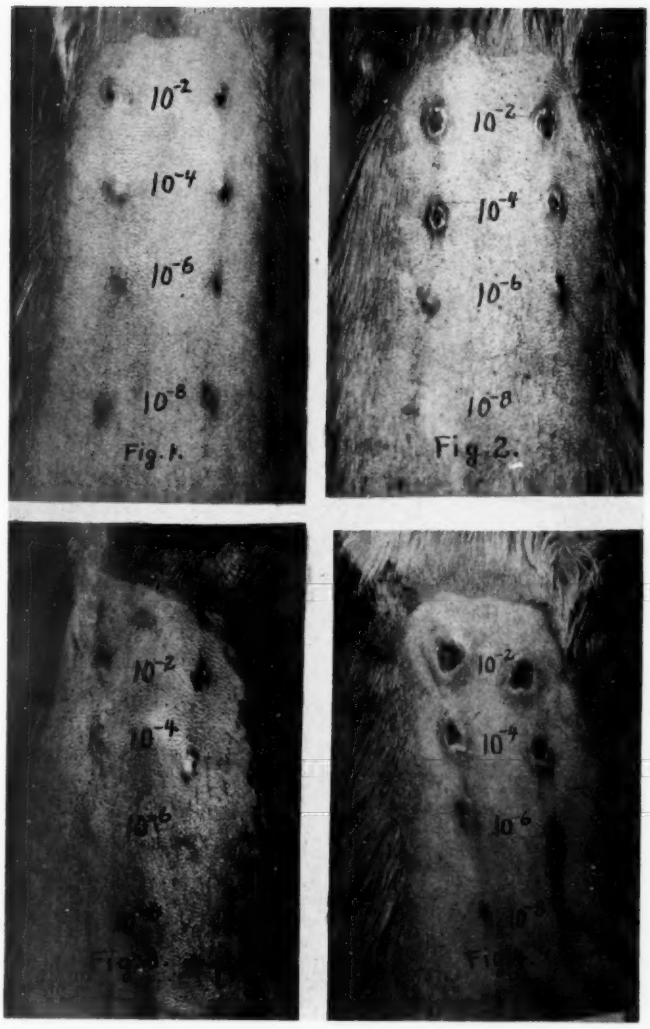

PLATE 1

Fig. 1. The lesions resulting one month after the intracutaneous injection of graded amounts (0.2 cc. of 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} mgm. per cc. in 5 per cent india ink) of virulent human tubercle bacilli (H7) in a guinea pig which had received a subcutaneous injection of 0.001 mgm. of the same strain of bacilli one month prior to the intracutaneous injections.

Fig. 2. The same as fig. 1 except that the subcutaneous injection consisted of 100 mgm. of heat-killed (in a boiling water-bath for 30 minutes) human tubercle bacilli (H7) instead of viable bacilli.

Fig. 3. This guinea pig received the same treatment as those shown in fig. 1, and this picture was taken four months after the intracutaneous injections (5 months after the subcutaneous injection of the viable bacilli).

Fig. 4. This guinea pig received the same injections as in fig. 2, and this picture was taken four months after the intracutaneous injections (five months after the subcutaneous injection of the heat-killed bacilli).

An examination of data recorded in a second table reveals that there are no striking differences in the findings in the intracutaneous sites of injection (totaling 0.004 mgm.) in the guinea pig markedly susceptible to the virulent human tubercle bacillus, regardless of whether the animal has been previously infected by a subcutaneous injection of 0.001 mgm. of viable virulent human tubercle bacilli or 100 mgm. of heat-killed (in a boiling water-bath for 30 minutes) human tubercle bacilli of the same strain. It must be remembered, however, that in both cases the criterion with culture is highly delicate and is capable of discerning the bacilli down to 0.000,000,01 mgm. in fine suspension, and that the intracutaneously infecting dose is relatively large. That this criterion did not fit the purpose of test in this case is readily realized when evaluating the gross tuberculous findings in the two cases (see figures) and considering the time of test (6 months). The guinea pigs given the killed bacilli show more extensive skin lesions at the sites of intracutaneous inoculation and in higher dilutions than those previously infected (one month prior) with viable virulent bacilli. Either the earlier first infection with viable bacilli had a retarding influence upon the intracutaneous lesions or the first injection of heat-killed bacilli (100 mgm.) had an enhancing effect upon the development of the lesions at the intracutaneous sites. The gross organic disease in the guinea pigs given the heat-killed bacilli subcutaneously one month prior to the intracutaneous infection was decidedly more marked than that found in the animals immunized with avirulent human tubercle bacilli. The gross organic disease in the guinea pigs which had been infected subcutaneously with 0.001 mgm. of viable virulent human tubercle bacilli (H7) one month prior to the intracutaneous injection was not a suitable companion for deductions.

THE FATE OF AVIRULENT HUMAN TUBERCLE BACILLI IN IMMUNE AND NORMAL GUINEA PIGS

An examination of the culture data recorded in a third table reveals that there are no practical differences in the positive findings for the large intracutaneous injections of the avirulent human tubercle bacilli when using a delicate criterion for testing. However, with this criterion there appears to be a more rapid disappearance of viable avirulent bacilli from the site of intracutaneous inoculation than when small numbers of bacilli have been injected in the normal as compared with the immune guinea pig. It is possible that a small number of viable bacilli have disseminated from the site to the local lymph nodes or to the internal

organs where they may be sparsely scattered. The local lymph nodes and lymphatic drainage centres unfortunately were not examined in this series because of the difficulty encountered in trying to examine all lymphoid and lymph nodes when injections were widespread over the skin as in these experiments.

In order to note what effect an injection of avirulent human tubercle bacilli would have on a subsequent intracutaneous injection of the same bacilli in terms of their dissemination, a series of guinea pigs were given a relatively small (for avirulent human tubercle bacilli) single intracutaneous injection (0.1 cc. of 0.1 mgm. per cc.) which does not produce organic deposition. One month later some of these guinea pigs were given another single intracutaneous injection of a larger amount (0.1 cc. of 10 mgm. per cc.) of the identical strain of bacilli which we hoped would show the differences in dissemination of the bacilli between a previously injected and a normal guinea pig providing such differences existed. At the same time, normal guinea pigs were given the larger intracutaneous injection as a control for the normal dissemination of these bacilli in this amount.

An analysis of the data recorded in a table indicates that the single first small intracutaneous injection of avirulent human tubercle bacilli, one month prior to a single second larger intracutaneous injection of the identical strain of bacilli, definitely retarded the dissemination of the bacilli from the larger second injection site of bacilli into the liver and spleen of the guinea pig, as determined by the delicate culture test, so that later positive findings as well as less decided positive findings occur in the previously inoculated guinea pigs as compared with the normal guinea pigs. (This experiment is now being repeated with a smaller second injection using 1 mgm. per cc. instead of 10 milligrams per cc., and will be reported subsequently.)

SUMMARY AND CONCLUSIONS

1. Delicate culture methods, suitable for determining small numbers of viable tubercle bacilli in tissues, reveal only slight differences between the findings in normal and immune guinea pigs injected with graded amounts of virulent human tubercle bacilli within the time available for experimental test in these highly susceptible animals. This can be understood when it is realized that the bacilli can remain viable in the body for months even in the absence of multiplication. However, in these same animals, there is a striking difference between the immune and

normal guinea pig in the amount of local and organic tuberculosis found.

2. Likewise, when virulent human tubercle bacilli are injected intracutaneously into guinea pigs which had been previously injected subcutaneously (one month prior) with heat-killed or viable virulent bacilli of the same strain, the culture results were not striking. The resultant skin lesions from graded intracutaneous injections are markedly more developed in the guinea pigs primarily injected with the dead (heat-killed) bacilli than they are in the animals given the primary injection of viable bacilli.

3. When avirulent human tubercle bacilli are injected intracutaneously into immune and normal guinea pigs, the culture findings reveal no striking differences in the larger injections within the time of experimental test possible. However, with the smaller injections, a decided retardation of dissemination of the bacilli from the intracutaneous site in the immune as compared with normal guinea pigs is to be noted. These findings are in complete accord with those obtained by Krause with virulent bacilli in immune and nonimmune guinea pigs.

4. When single large doses of avirulent human tubercle bacilli are injected intracutaneously into normal and immune guinea pigs, there is also noted a definite retardation of deposition of the bacilli in the internal organs (liver and spleen) in the immune as compared with the normal guinea pigs. The differences are relative and not absolute with the large doses necessarily used in these tests.

We are grateful to L. D. Miller for assisting with the technical work reported in this study.

REFERENCES

These will be found at the end of Study V, where all references for the whole series are assembled.

STUDIES ON THE BEHAVIOR OF TUBERCLE BACILLI WITHIN THE BODY

III. THE LOCAL CUTANEOUS REACTION TO VIRULENT TUBERCLE BACILLI IN GUINEA PIGS

H. J. CORPER,[1] A. P. DAMEROW[1] AND MAURICE L. COHN[1]

The guinea pig is ideally suited for experiments in tuberculosis, and, although the results in this animal have variously been considered inconsequential by some for the interpretation of tuberculosis as it occurs in man, it must be admitted even by the most critical of those condemning a direct translation of results, that there are some striking similarities. For instance, the pathological cellular reactions from acute tubercle to fibrosis and caseation, when properly evaluated, resemble those seen in man; the specific tuberculin reaction shows a striking similarity, now that we acknowledge a close relationship between protein anaphylaxis and allergy; and, barring organic differences and forced unnatural experiments in a highly susceptible animal, proper and graded experiments show striking similarities when correctly evaluated.

In order to verify earlier observations in guinea pigs and to discern the significance of one infection with the same tubercle bacilli (to rule out possibilities of strain and specific reactions as far as can be done) upon previous and subsequent infections with the same bacilli in single or separate double injections given at different intervals, guinea pigs were given intracutaneous injections of viable virulent bacilli of human and bovine strains. The intracutaneous injections were given preference because they could be utilized for repeated observations in the living animal, and the same animal could be studied for the significance of repeated injections with virulent bacilli. It was also possible to duplicate suspensions of the tubercle bacilli both as to the amounts used for injection and the age of culture at the time of use (Paper I, this series). Krause (7) (25) has already shown differences on repeated single injections with heavy suspensions of human tubercle bacilli. It appeared, however, essential as a basis for further studies being carried on by us,

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to undertake in greater detail investigations along this same line with virulent human and bovine tubercle bacilli.

For the purpose of determining the effect of the first injection upon subsequent injections as well as the presence of retroactive effect, and also to note whether differences occurred when coincident injections were single or double, or whether the time interval played a part, a series of guinea pigs were divided into groups,—some receiving single intracutaneous injections of fine suspensions of virulent bacilli and some coincident double injections at semimonthly and monthly intervals. Three different strains of virulent tubercle bacilli (two human,—Gluckson and sputum 7, and one bovine,—Bovine virulent) were used for separate sets of animals. All injections were given intracutaneously, and to the suspension was added a small amount of india ink (about 1 per cent) to serve as a marker for the site of injection (Paper I, this series). The injections were carefully given, using a graduated tuberculin syringe and a short bevel 27-gauge $\frac{1}{2}$-inch hypodermic needle. The amount injected was 0.2 cc. and back leakage was carefully guarded against. All lesions were read at weekly or semiweekly intervals by measuring the lesions as nearly as could be done, and at definite intervals photographic records were made to the same magnification. Since the differences in the strains, whether human or bovine, used in the guinea pig for the purpose of the study outlined above resolved themselves rather into relative differences in findings, dependent upon the grade of virulence instead of on the species or strain of bacilli, it did not seem pertinent in presenting the results that all the detailed animal data be given, but that sufficient description of a classical composite result in the guinea pig be presented, together with a few illustrative photographs. The experimental part itself was performed on duplicate or triplicate animals for each type of test and sufficient controls to guarantee absence of possible errors arising from the use of suspensions made at different times, although previous tests had ensured the ability to reproduce these suspensions in age of cultures, quantity of bacilli, and state of division. Differences in the age of the animal within the experimental period did not appear to be of sufficient consequence to alter the general findings, although this was also controlled by using animals of slightly different ages in the control first-injection tests for the suspensions at the different stages of the experiment. Decided differences can be noted for large injections, as already recorded by Krause and conforming to the classical Koch phenomenon. However, when the amounts given as second and subsequent

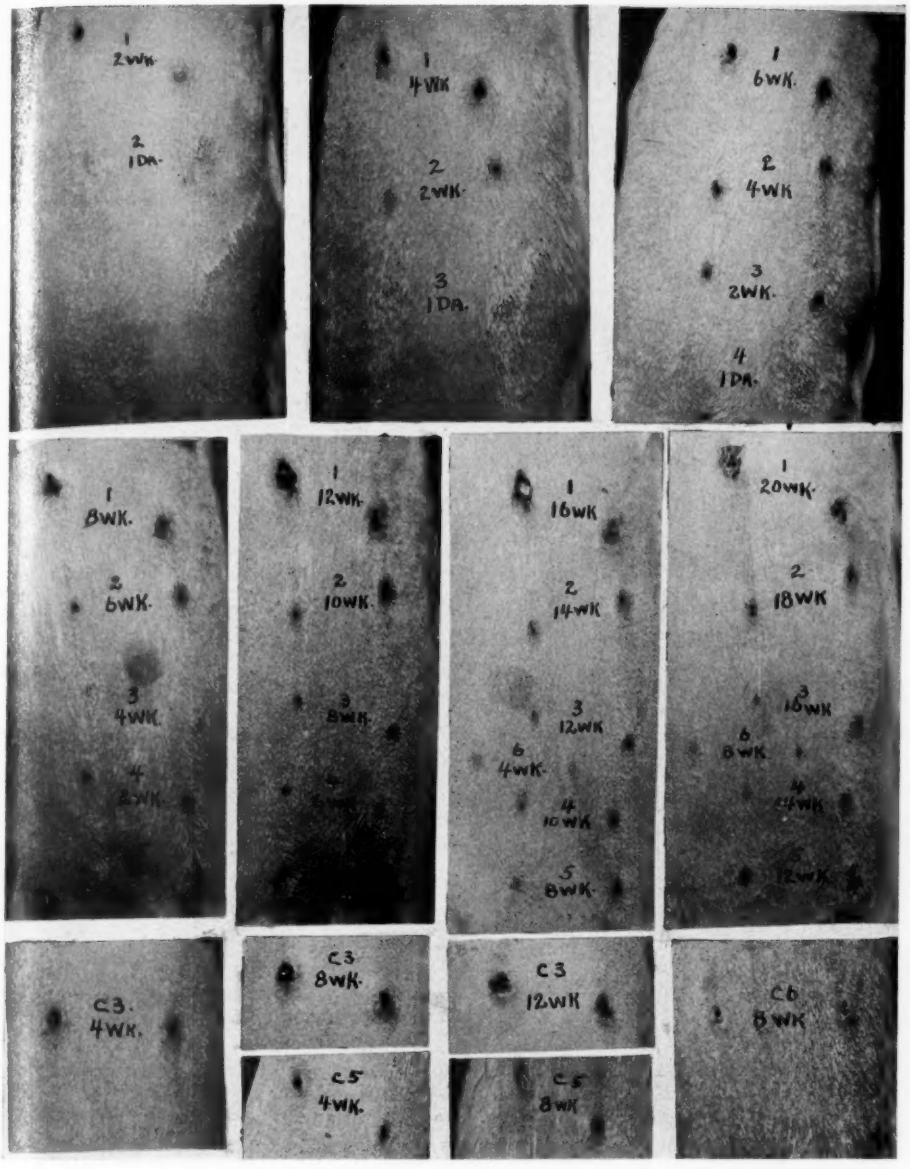

PLATE 1

Results of the duplicate intracutaneous injection at semimonthly intervals of 0.2 cc. of 0.001 mgm. per cc. (with india ink) of a virulent laboratory strain of human tubercle bacilli (Gluckson) into the guinea pig. Note the progression of the first lesions 1 at intervals up to 20 weeks after injection and the retardation of all subsequent lesions 2 to 6 inclusive. Note also in the lower row of pictures the progression of the control injections C3, C5, and C6 following the intracutaneous injection of the same suspension into a normal guinea pig corresponding to the similarly numbered lesions 3, 5, and 6 of the original repeatedly injected animal. The interval after each injection is recorded in weeks (*wk*) beside the lesion pictured. Note the erythematous zone around the 1-day sites of injection in the three top pictures which usually subsided in several days to a week and is not found until after the first injection.

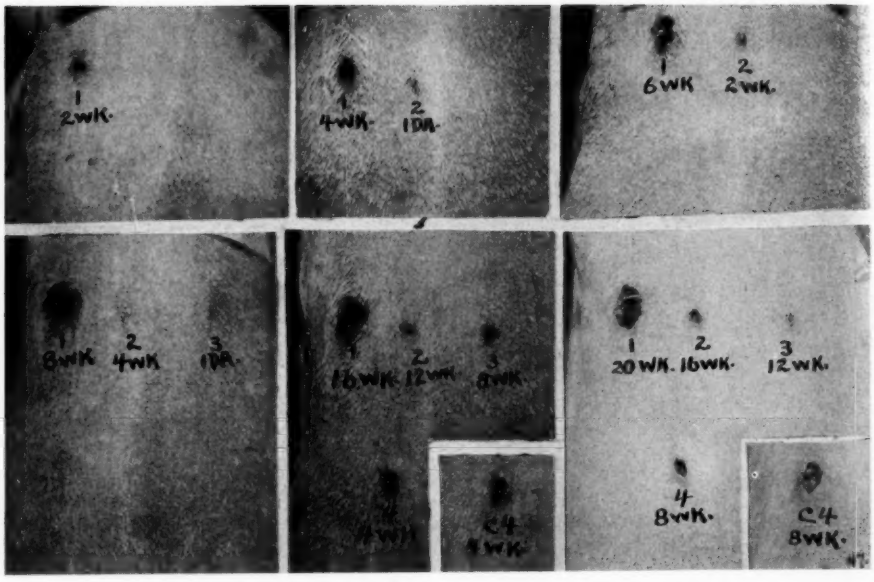

PLATE 2

Results of single intracutaneous injections at monthly intervals of 0.2 cc. of 0.001 mgm. per cc. (with india ink) of a recently isolated (from sputum) virulent strain of human tubercle bacilli (sputum 7) into the guinea pig. Note the progression of the first lesion and the absence of tendency to heal, up to 20 weeks. These lesions are easily injured and torn in handling the animal, which accounts for the ragged appearance of the "1" lesion in some of the pictures. The initial erythema and oedema is noticeable 1 day after injection in the second and subsequent lesions. Note the progression of the control C4 (insert picture) at the 4 and 8 weeks' interval in a normal animal, as compared with that in the previously injected animal site "4" at the 4 and 8 weeks' with the same suspension. Note also that the "4" injection has developed slightly more than the "2" and "3" sites,—perhaps as a result of the generalization of the disease in the animal at this time.

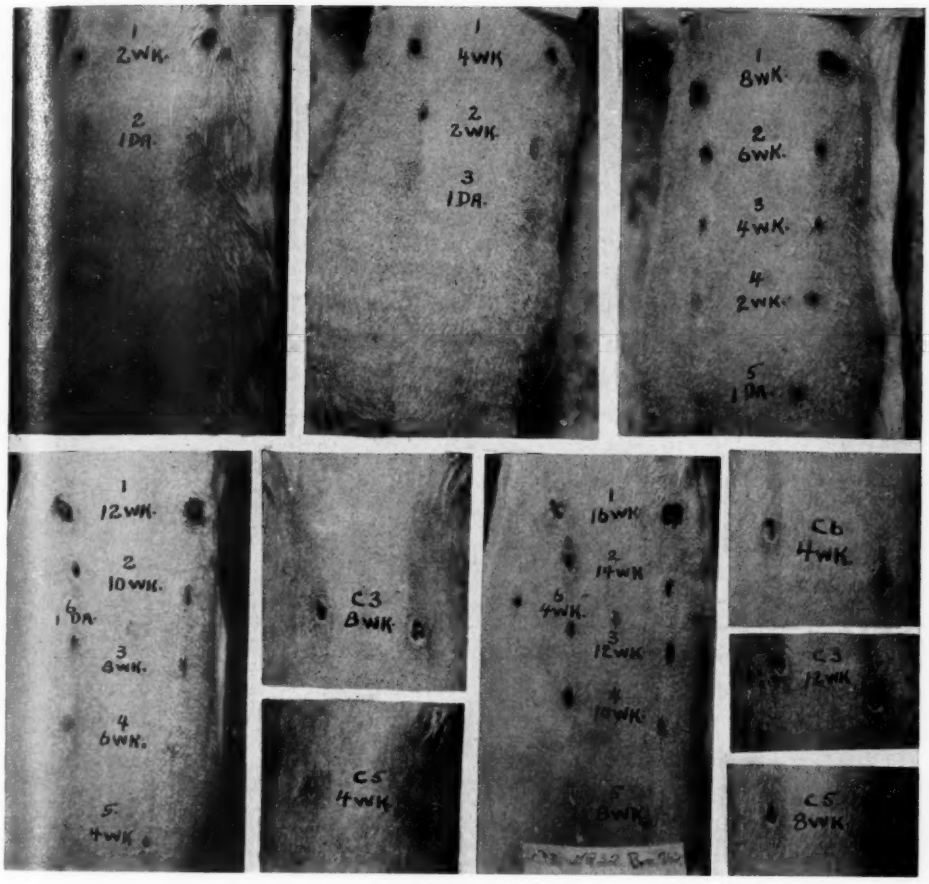

PLATE 3

Results of duplicate intracutaneous injection at semimonthly intervals of 0.2 cc. of 0.001 mgm. per cc. of a virulent strain of bovine tubercle bacilli (Bovine virulent) into the guinea pig. This strain proved more pathogenic to guinea pigs than either of the human strains used. Note the decided erythema and oedema 1 day after the 2d and 3d injections of bacilli. Note also the more decided progression of the initial injections as compared with the later ones as well as the progression in the controls in normal animals injected with the same suspension (C3, C5, and C6) corresponding to the same numeral (3, 5, and 6).

injection are small (about 0.000,001 mgm.), there may result a complete absence of all phenomena, including the allergic local reaction as compared with the control, and the development of no lesion at the site of injection in spite of the fact that one-millionth of a milligram will infect a normal guinea pig by this route, cause a skin lesion, and finally result in generalized organic disease. With intermediate amounts, however (0.001 mgm.) the effects approximate the heavier injections, but the retardation in the development of the local lesion is striking. As the development of the lesion in the control animals resembles that caused by the first injection, it will suffice to describe approximately the lesions as they develop at weekly or biweekly intervals. Since the lesions develop similarly, regardless of whether the intracutaneous injections in the same amount are single or double or whether the injections are given at semimonthly or monthly intervals, a typical course of events in an animal given semimonthly intracutaneous injections will be presented.

The following description conforms to the findings for a typical virulent human strain in the amount of 0.2 cc. of a 0.001 mgm. of bacilli per cubic centimetre of fine suspension. For the first intracutaneous injection there was present at one week a definite elevated hard nodule about 4 mm. in diameter; at two weeks this nodule had further developed in size to about 6 mm. in diameter, which may or may not ulcerate to result in a 2- to 3-mm. discharging ulcer; at one month the ulcer had enlarged to about 4 mm. on an 8-mm. lesion base; at six weeks the ulcer had enlarged to 5 to 7 mm. on a 10-mm. lesion base; at two months the ulcer had slightly enlarged to about 6 to 8 mm. on a 12-mm. lesion base; at three months the lesions still were ulcerating and active, although there was some tendency toward healing and scar-tissue formation; at five months, if the animals were still alive, the ulcer was still present, although in some cases there was a definite tendency to heal.

For second intracutaneous injections given two weeks after the first, there occurred a decided erythema varying in extent from 5 to 10 mm. in diameter on the first day following the injection of the 0.001-mgm. concentration (this may be lacking with high dilutions of bacilli), which persisted as long as a week in some animals; after one week, a barely perceptible nodule was evident; after two weeks, this nodule may enlarge to 2 to 4 mm. in diameter; after four weeks this nodule may enlarge to 4 to 6 mm. in diameter and occasionally rupture, to produce a small ulcer about 1 to 2 mm. in diameter; after six weeks the lesion remained about the same as at four weeks; after eight weeks there was a decided

tendency to heal; after three months retrogression was evident and ulcers, if developed, tended to heal.

The third semimonthly intracutaneous injection given one month after the first tended to follow even a slightly more benign course than the second injection, while a control test of the same suspension and with the same amount in a normal animal tended to pursue the course noted for the first injection. Likewise, the fourth and fifth semimonthly intracutaneous injections followed a benign course similar to the second and third intracutaneous injections. The sixth semimonthly injection, although not as progressive as the first, tended to be slightly more progressive in some animals than the third and subsequent intracutaneous infections. Coincident with this effect, there was found at autopsy of some of these guinea pigs the presence of a generalized advanced organic disease, probably resulting from the first intracutaneous inoculation of the bacilli.

DISCUSSION

It is evident from these observations that even in the highly susceptible guinea pig there is a definite retardation to the development of the local lesion following the intracutaneous injection of virulent bacilli in small and relatively large amounts in consecutive single or double injections given at semimonthly or monthly intervals. This is in conformity with the findings by Krause for the same route of infection and for the same animal, but it extends the observations to both human and bovine bacilli and to graded amounts of bacilli. It also verifies the contentions of many other observers, who have pointed out the existence of a protective action of a previous infection with virulent tubercle bacilli. Among these may be included in addition to Krause, particularly Koch, Trudeau, Baldwin, Webb, Sewall, Petroff, Calmette, Park, and others. It is also evident from these observations that desensitization plays no part in the progression of the lesion with virulent bacilli since the first site of intracutaneous injection of the virulent bacilli becomes more extensive in each case than the subsequent injections of the same amount, while in the latter case sensitization is pronounced, as is shown by local erythematous reaction but is absent with the first injection. Whether the sensitization is the important part of the immune reaction or not might be doubted from the fact shown in a previous paper (Paper II, this series) that heat-killed (boiling water-bath) bacilli showed no retardation of the lesions as compared with infection with viable bacilli.

With small infections (approximating 0.000, 001 mgm.) the retardation of development of lesions is much more striking, so that no visible tissue reaction is discernible in the previously injected animal as compared with the control in which there results a chronological sequence of events leading to definite lesion-formation and its consequences. It appears obvious that in man the natural heavy infections must be unusual as compared with the repeated small spontaneous infections, so that immunity should play an important rôle in such infections.

Coincident double injections do not affect each other as to the difference of development of their lesions as compared with single injections, and later injections do not have a retroactive effect on the further development of previous injections at the original site. The immune effect requires time to develop and apparently is active only against new plants and new extensions.

SUMMARY

1. There is a definite chronological development of the tuberculous skin lesion following the first intracutaneous injection of virulent human or bovine tubercle bacilli in the guinea pig, dependent upon the amount of bacilli and their relative virulence for this animal. The first lesion develops when as little as 0.000,001 mgm. of these virulent strains are injected intracutaneously.

2. The chronological sequence of development of the tuberculous skin lesion following the second and subsequent intracutaneous semimonthly injections of the same virulent human or bovine tubercle bacilli and in the same quantity shows a definite retardation of these lesions.

3. With smaller semimonthly injections there may occur in the second and following infection a complete absence of reaction or lesion at the site of intracutaneous injection as compared with the normal animal.

4. The protective effect, which increases gradually, requires a certain interval of time to develop and exerts no apparent retroactive effect on the first previously established lesions except apparently as they tend to produce new plants and extensions in normal tissues.

Valuable assistance in performing these experiments was rendered by Catherine Clark.

REFERENCES

The References will be found at the end of Study V, where those for all five papers are assembled.

STUDIES ON THE BEHAVIOR OF TUBERCLE BACILLI WITHIN THE BODY

IV. THE CUTANEOUS REACTION TO AVIRULENT TUBERCLE BACILLI AND IMMUNITY

H. J. CORPER,[1] M. L. COHN[1] AND A. P. DAMEROW[1]

In previous studies (Paper III, this series) it was shown that there is conferred upon animals by the first infection with virulent tubercle bacilli a protection against subsequent infections, verifying earlier observations and opinions expressed by other observers (26). This protection contributed by the first virulent infection is relative rather than absolute and is dependent upon the subsequent infecting doses. With small doses, evidences of tuberculosis developing from the second and subsequent infections could be lacking in the animal immune to reinfection (Paper III, this series).

It is obvious that such experiments are complicated by the tuberculosis developed from the first infection and leave little more to be accomplished from a practical standpoint (23). This is especially true for interpretation in a quantitative way in an animal highly susceptible to the organism in question unless these results can be duplicated by means of nonviable or otherwise innocuous materials, or materials which rule out confusion between it and the organisms used. Calmette was one of the first who stressed the existence of tubercle bacilli in avirulent forms by introducing the avirulent bovine tubercle bacillus (*Bacillus Calmette-Guérin*), and yet the criterion for avirulence used by him left doubt as to the existence of a stable avirulent bacillus within the realms of practical interpretation. This instability was contended to exist by certain investigators, who believed that they could isolate by culture methods virulent and avirulent strains of BCG or that a marked instability existed in the progeny of not many generations removed in cultures of BCG. Yet their criteria were lacking in conclusiveness. For this reason certain fundamental information is essential to avoid the confusion of virulence and avirulence of tubercle bacilli.

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Although no arbitrary exclusive definition can be given for tubercle bacilli, there are certain differential characteristics for the acid-fast saprophytes, the so-called pathogens (parasites) for cold-blooded animals, and the pathogens (parasites) for warm-blooded animals. The latter will not grow except within a narrow range of temperature, approximating 37°C., and then only slowly, as is particularly evident for human and bovine tubercle bacilli. In spite of the fact that pathogenicity may change during prolonged artificial cultivation, the optimum temperature requirement for growth remains stable, so that this criterion can be used to differentiate the tubercle bacilli (progeny of human and bovine strains) from the other usual acid-fasts (saprophytes and parasites for cold-blooded animals) encountered. There is, however, a remarkable stability in the virulence of the same culture that has remained viable in vitro for a long period of time (15). Avirulent bacilli residing in the dog for over three years do not become virulent. In an earlier study (Paper I, this series) it was shown that tubercle bacilli, avirulent or apathogenic for an animal species, when injected intracutaneously in small amounts have a definite tendency to disappear in viable form from the site of inoculation in that species. Universally avirulent human tubercle bacilli (apathogenic to all animal species) also disappear in viable form from the intracutaneous site of inoculation and possess little tendency to disseminate to internal organs. Virulent tubercle bacilli for the species in question tend to multiply at the site of inoculation, as well as the site of dissemination to the organs, unless the amount inoculated is too small to produce ultimate tuberculous changes, when they also tend to disappear in viable form from the site of intracutaneous inoculation and of dissemination. There is a definite quantitative relation between pathogenicity and virulence in the susceptible species to a specific virulent strain. It would appear, therefore, that the criterion of virulence is a criterion of ability of the bacilli in question to multiply in the host in question, and that avirulent bacilli possess no power to multiply in the host within reason of practical quantitative test. From this it follows that an overwhelming dose of bacilli can hardly be used as a just criterion to determine virulence and would lead to misinterpretation and gross error. It is well known that a large injection of dead or avirulent bacilli can produce marked tuberculous destructive changes together with general symptoms and even ultimate death of the animal. For this reason an attempt was made to establish a practical criterion for avirulence. After due consideration on the basis of earlier experiences with infecting

animals and growing tubercle bacilli, it appeared rational that, if avirulent bacilli could not multiply in the host, the reaction to them on injection, if this could be satisfactorily measured, should parallel that obtained with equal amounts of dead bacilli. Since the manner of killing the bacilli may be a factor in the host's reaction to them, and since there also might be introduced the factor of chemical reaction when chemicals were used for destroying the viability, a number of substances were tried for this purpose. To obviate further individual animal reactions, a number of comparisons were also made in the same animal, using graded injections and viable bacilli. A large number of substances and treatments were tried, including heat (boiling-water temperature for 30 minutes), hexylresorcinol, phenol, acetic acid,[2] and others (1). When a compound produced a local tissue injury in the concentration used for killing the bacilli, the method of neutralization or dilution to a point of being innocuous was used to prevent such action after the bacilli had been killed; for example, to prevent the toxic action of acetic acid on the tissues it was neutralized with sodium hydroxide, while hexylresorcinol and phenol were diluted. At the same time, culture controls of all such suspensions proved the bacilli nonviable. Such experiments were used to test three avirulent strains of tubercle bacilli available to us. Two of these were human,—a human avirulent and "Bernstein" (previously described (15)), and the third was the attenuated bovine, BCG, obtained from Drs. Wm. H. Park and Lucy Mishulow. Briefly, although slight variations occurred in the individual animals, the course of the lesion resulting from the intracutaneous injection of a similar amount (0.1 to 0.2 cc.) of fine suspension of the same strain of dead bacilli (heat or chemically killed) and viable bacilli of these three strains was almost identical. The bacilli were injected in graded amounts, ranging from 10 to 0.01 mgm. per cubic centimetre. The results in typical cases are illustrated in the figures. Although no striking differences between the reaction to the three avirulent strains was noted, BCG at times appeared to produce a more acute toxic effect which was evident by the immediate larger local lesions and ulcers with the viable bacilli.

From these results the question naturally arose whether there was any striking difference between the local effect of dead virulent human or bovine bacilli as compared with the dead avirulent bacilli. In order to

[2] In fine suspension in heavy concentrations of 0.9 per cent sodium chloride, tubercle bacilli are destroyed within 30 minutes to one hour at 37°C. by equal volumes of 5 per cent acetic acid.

determine this, two strains of virulent bacilli (one human—7, and one bovine—Bovine virulent) were compared with the avirulent human and BCG. The amounts injected intracutaneously ranged from 10 to 0.01 mgm. per cubic centimetre (0.1 or 0.2 cc. being injected into the individual animal), using the same reagent for destroying the viability of the bacilli to be injected into the same normal animal. As above, the chemicals used in these tests were acetic acid, hexylresorcinol, and phenol. Within the limits of such a test, there was found to be no difference between the reaction developing to the dead virulent bacilli as

PLATE 1

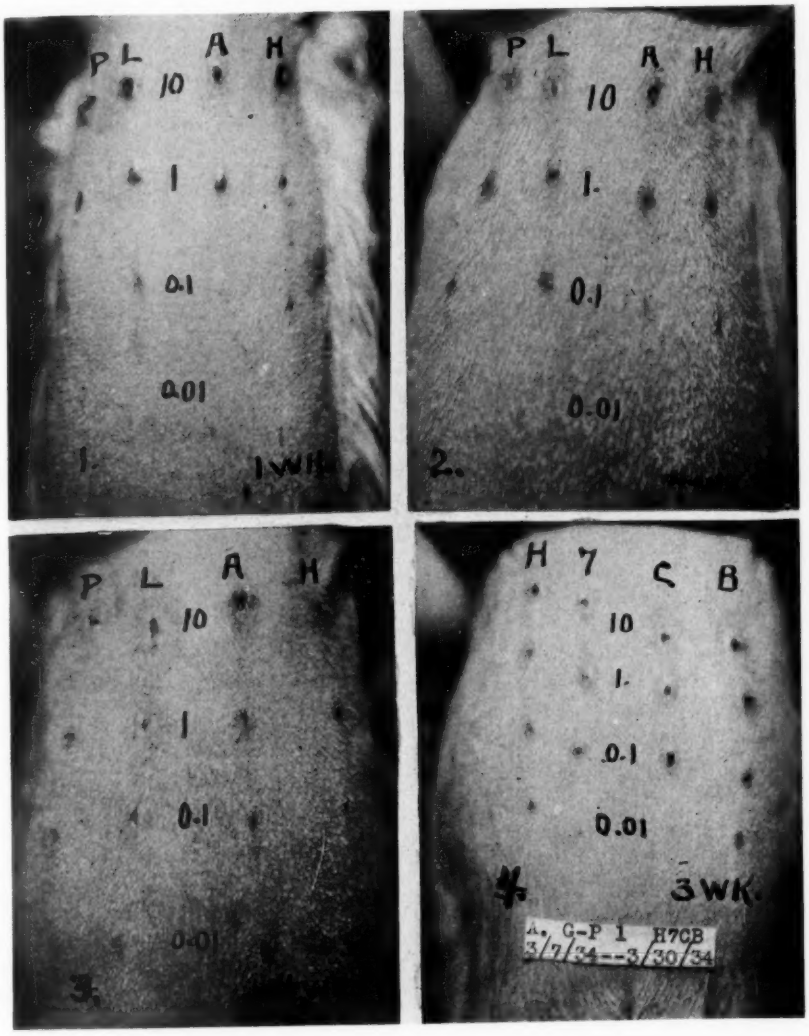
Fig. 1. Results of the intracutaneous injection into the guinea pig of 0.2 cc. of different amounts (10, 1.0, 0.1, and 0.01 mgm. per cc.) of a fine suspension of dead and alive avirulent human tubercle bacilli (Human avirulent). In the vertical column "P", the bacilli were killed with phenol; "A", with acetic acid; and "H", with hexylresorcinol; while the live bacilli for comparison were injected in the row designated by column "L". The interval illustrated is one week after injection. Note the approximate similarity of the lesions produced by the same amount of injection (horizontal rows) of viable and nonviable avirulent human bacilli.

Fig. 2. The same animals illustrated four weeks after injection. Note retrogression and healing of lesions at this time.

Fig. 3. This animal received the same treatment as that in fig. 1, except that avirulent bovine tubercle bacilli (BCG) were used in place of the avirulent human tubercle bacilli. The illustration is four weeks after injection.

Fig. 4. Results of the intracutaneous injection into the guinea pig of 0.2 cc. of different amounts (10, 1.0, 0.1, and 0.01 mgm. per cc.) of a fine suspension of dead avirulent human ("H"), avirulent bovine BCG ("C"), virulent human ("7"), and virulent bovine ("B") tubercle bacilli. These bacilli were made nonviable by treatment with an equal volume of 5 per cent acetic acid for two hours at 37°C. and the acetic acid neutralized with sodium hydroxide. Note the similarity in the lesions resulting from the injection of the same amounts of bacilli (horizontal rows), regardless whether avirulent or virulent, human or bovine bacilli were used. The photograph was taken three weeks after injection. The same comparative results were obtained when the bacilli had been made nonviable with phenol and hexylresorcinol in place of the acetic acid.

compared with the dead avirulent bacilli, and between the dead human and dead bovine tubercle bacilli. This is well shown in the figure illustration. From these results and culture tests for viability previously recorded (Paper I, this series), it appears highly improbable that a multiplication of these avirulent tubercle bacilli occurs in the animal body. From this it would hardly appear possible that dissociation, as reported for cultures (27) (28) into virulent forms from avirulent forms, could take place in the animal. It is also doubted whether there is available to-day reliable and indisputable evidence of the transmutation


P L A H
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H 7 C B
10
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4
3 WK.
A, G-P 1 H7CB
3/7/34--3/30/34

PLATE 1

or dissociation of avirulent to virulent tubercle bacilli by animal passage alone. These experiments, in our experience, are self-limited and self-extinguishing without resort to culture intervention because there is a rapid dilution of bacilli in passage from one animal to another even when initiating the test with relatively large amounts of bacilli. We are inclined to agree with Willis (29) "that the prolonged stay of a weakly virulent tubercle bacillus in animal tissues does not affect the virulence as a rule."

CUTANEOUS REACTION TO AVIRULENT TUBERCLE BACILLI

It is pertinent that we fully understand the tissue reactions in a normal animal to various doses of tubercle bacilli. Yet, when these bacilli can

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PLATE 2

Figs. 1 to 5. The development of lesions in the dog following the intracutaneous injection (in duplicate) of graded amounts (0.2 cc. of 10 to 0.000,1 mgm. per cc.) of viable avirulent human tubercle bacilli (Human avirulent) in fine suspension. Note the early oedematous reaction and later development of undermined ulcers in higher concentrations, with gross lesions in concentrations as low as 0.01 mgm. Note also the regression and healing after six to ten weeks.

Figs. 6 to 10. These show the development and healing of the lesions in the rabbit following the same intracutaneous injections of graded amounts of avirulent human tubercle bacilli as illustrated in figs. 1 to 5 for the dog.

Figs. 11 to 15. The results of the intracutaneous injection into the dog of graded amounts (0.2 cc. of 10 to 0.000,1 mgm. per cc.) of viable avirulent bovine tubercle bacilli (BCG). Note the course of the infection and the similarity between these lesions and those in the dog (figs. 1 to 5) injected similarly with viable avirulent human tubercle bacilli.

Figs. 16 to 20. These show the development and resolution of the lesions in the rabbits given intracutaneous injections of BCG similar to the dog illustrated in figs. 11 to 15.

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multiply in the animal, there immediately arises the question of changed effects due to continually changing numbers of bacilli. Although killed bacilli may be used, there has always entered into the interpretations the problem of the effect of the agent used for this purpose and the chemical change produced. Since it is fairly certain that avirulent bacilli, at least in reasonable amounts, do not possess the ability to multiply in the tissues it appeared that tests with graded amounts of avirulent tubercle bacilli in different animal species would yield valuable control data upon which the interpretation of other tests might depend. It is notable that much of the testing with BCG has been done without regard to amounts of bacilli (30). For the purpose of tests, guinea pigs, rabbits, and dogs were given intracutaneous injections of varying amounts (0.2 cc. of 10,

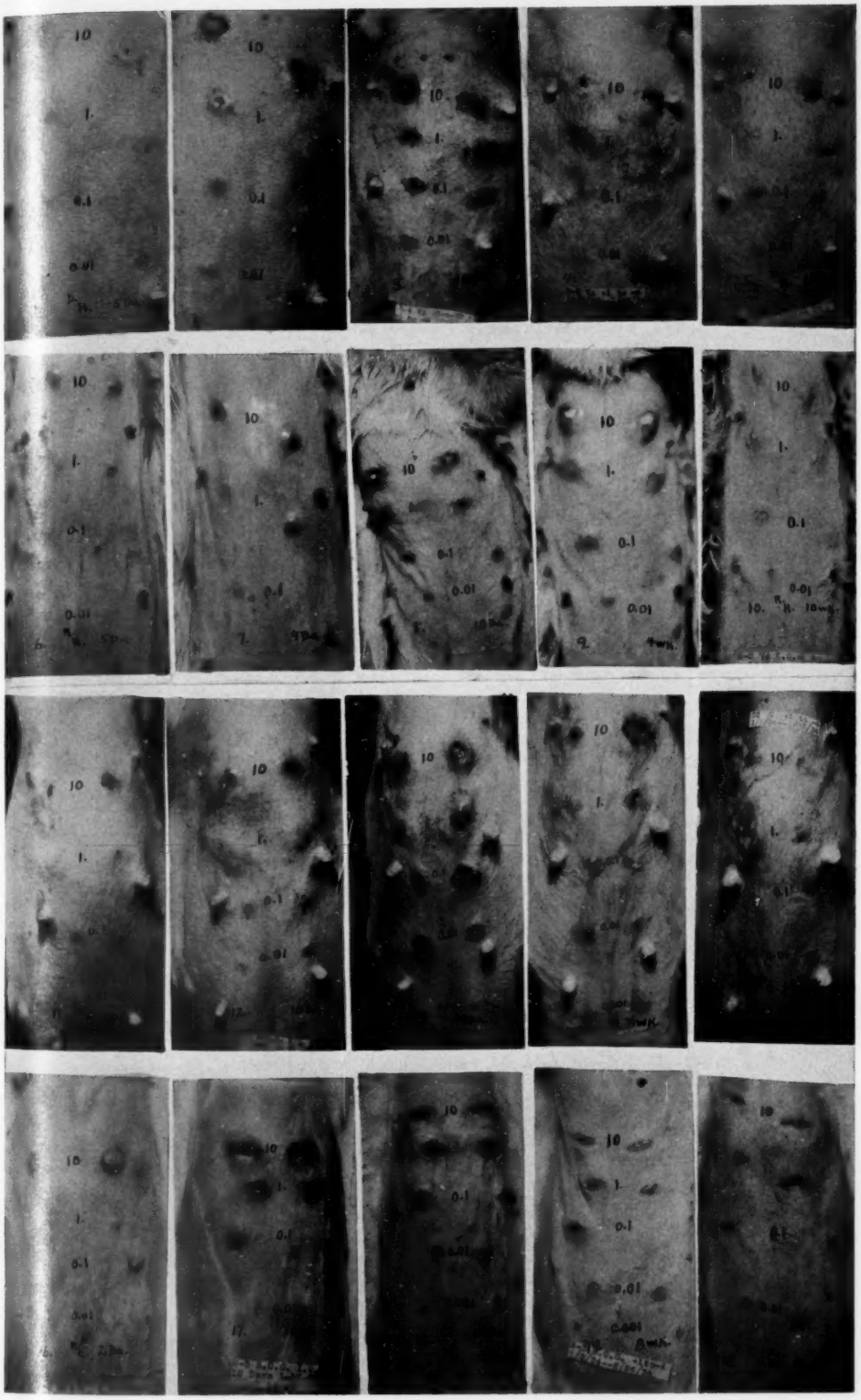

PLATE 2

1.0, 0.1, 0.01, 0.001, and 0.000,1 mgm. per cc.) of fine suspensions of avirulent human (Human avirulent) or avirulent bovine (BCG) tubercle bacilli. These bacilli were not virulent for any of these species of animals tested because they were unable to multiply in the tissues and therefore produced no metastases from the original sites of injection, so that no lesions developed in the internal organs from these injections. In the guinea pig, an ulcerative lesion was produced with the larger amounts (10 and 1 mgm. per cc.) of both the avirulent human and bovine (BCG) bacilli. These ulcers developed within the first week after injection and after discharge tended to heal gradually. Coincidently, there occurred slight local adenopathy which also tended to gradual resolution. Macroscopic lesions developed at the site of injection down to the 0.01 mgm. per cc. concentration, and with the human bacilli only a small transient nodule was found, which may or may not ulcerate. With the bovine bacilli, a gross lesion occasionally occurred with the 0.001-mgm. concentration.

The results with the dog and rabbit, although showing slight variations for the individual animals, were generally similar to those in the guinea pig, although the lesions as a whole were decidedly larger in the larger animals. The reaction in the dog was more exudative in the early periods than in the rabbits for the larger injections, but the resulting ulcerations were strikingly similar. The bacilli produced lesions (nodules) in both animals down to the 0.01- or 0.001-mgm. concentration. For these animals, BCG was slightly more toxic than the avirulent human strain. The maximum effect was consummated at about two to three weeks after injection. The illustrations show the development and regression of typical lesions in the dog and rabbit.

CUTANEOUS IMMUNITY PRODUCED BY AVIRULENT TUBERCLE BACILLI

Apparently from the foregoing experiments avirulent human and bovine tubercle bacilli can in reasonable dosage show no evidences of producing a progressive disease beyond the lesions caused by the same nonviable bacilli. It, therefore, becomes pertinent to determine whether these avirulent tubercle bacilli can produce the same immune effects previously noted for the virulent tubercle bacilli (Paper III, this series). The results of intravenous injections of these avirulent tubercle bacilli will be recorded in another paper, but it suffices to say here that even large amounts of fine suspensions given intravenously did not produce the characteristics of a progressive tuberculosis in guinea pigs in rea-

sonably large amounts (1 mgm.), while the addition of 0.000,001 mgm. of a virulent strain produced a generalized progressive disease in these animals equal to that produced when the virulent bacilli were given intravenously.

The following experiments were performed in guinea pigs to determine the effect of the injection of avirulent tubercle bacilli upon subsequent intracutaneous infections with virulent tubercle bacilli. In the first experiment guinea pigs were given intracutaneous injections of avirulent human (Human avirulent) or avirulent bovine (BCG) tubercle bacilli. In all the experiments recorded in this study, at least three to five male guinea pigs were used for each individual test, and they were chosen of approximately the same age and size. Two sets of first injections with avirulent tubercle bacilli in different doses were given. To assure satisfactory immunization, the pigs were given double or single intracutaneous injections of 0.2 cc. of a suspension containing 10 mgm. per cc. (large immunizing dose) or 0.2 cc. of 0.01 mgm. per cc. (small immunizing dose). From one to three months (differing for the individual sets of immunized animals) (after the first avirulent intracutaneous injection) all of the immunized and eight normal control guinea pigs for each type of infection were subjected to a single or a double intracutaneous infection with 0.2 cc. of a fine suspension of virulent human tubercle bacilli (H7), containing 0.001 mgm. per cc., or in other cases graded amounts of a fine suspension containing 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} mgm. per cc. The development of the lesions were read at weekly intervals, and photographic records made of many of them at intervals so that the sequence could be followed. In practically all cases in which the large intracutaneous immunizing injection with avirulent bacilli was given, there resulted a decided retardation in the development of the lesions resulting from the single or double intracutaneous injection of the virulent bacilli, and also in the lesions resulting from the graded injections from 10^{-2} to 10^{-8} which expressed itself as an absence of lesions or retardation in the higher dilutions. With the small immunizing dose the retardation was not quite as striking, although still evident. The interval between one and three months following the first avirulent injection, after which virulent infection occurred, did not play a striking rôle although the longer interval (three months) appeared to disclose a greater effect in retarding the virulent infection. The illustration depicts typical animals with their controls following the single intracutaneous injection of the virulent bacilli.

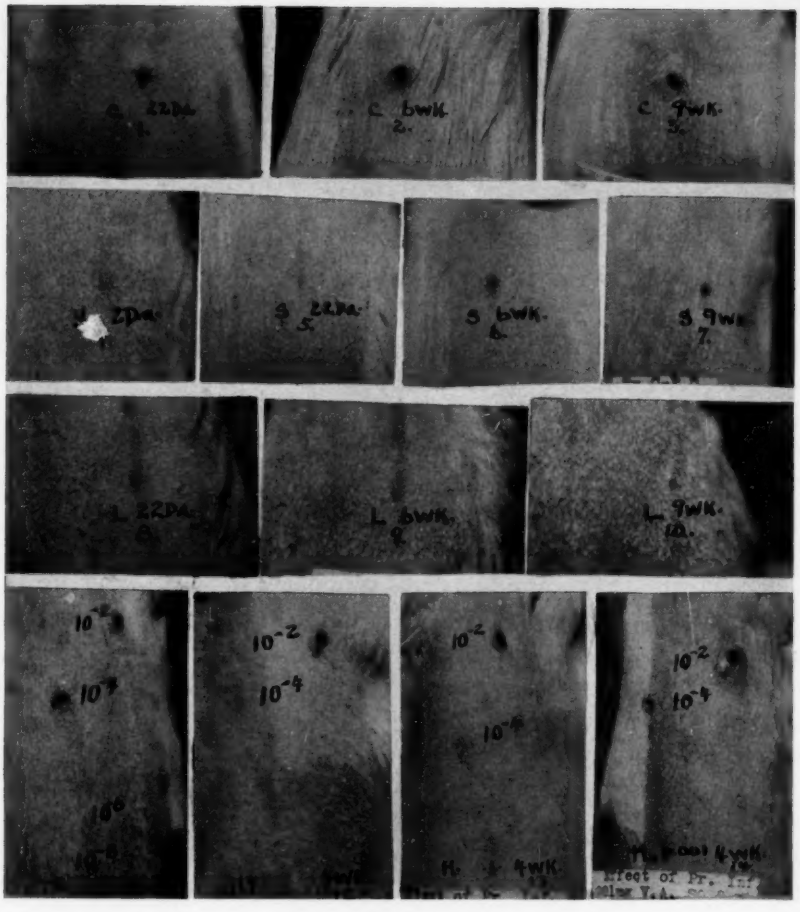

PLATE 3

Figs. 1, 2 & 3 (first row) illustrate the development of the lesions following the intracutaneous injection of 0.2 cc. of 0.001 mgm. per cc. of fine suspension of virulent human tubercle bacilli (H7) in a normal guinea pig (C).

Figs. 4, 5, 6 & 7 (second row) illustrate the development of the lesion following the single intracutaneous injection of the same bacilli given to the guinea pig (S) illustrated in figs. 1, 2 & 3. The animal had received a small (S) intracutaneous injection of avirulent human tubercle bacilli about two months prior to the virulent infection. Note development of erythema around site of injection at two days and retardation in development of lesion subsequently as compared with that in the control normal guinea pig.

Figs. 8, 9 & 10 (third row) show the development of the lesion in the guinea pig (L) given the same intracutaneous injection with virulent bacilli as the "C" and "S" animals, but given large intracutaneous injections of avirulent human tubercle bacilli three months prior to virulent infection. Note almost complete absence of lesion in this animal.

Figs. 11 to 14 illustrate guinea pigs given the same staggered intracutaneous injection of 0.2 cc. of graded amounts (10^{-2} to 10^{-8} mgm. per cc.) of fine suspensions of virulent human tubercle bacilli (H7) one month after the subcutaneous injection of "C" (fig. 11) the control; H7 (fig. 12) received 0.001 mgm. of virulent human tubercle bacilli (H7); H-1 (fig. 13) received 1 mgm. of avirulent human tubercle bacilli (Human avirulent); H-0.001 (fig. 14) received 0.001 mgm. of avirulent human tubercle bacilli. Note the retardation in the development of the 10^{-4} lesion in both the H-7 (fig. 12) virulent bacillus immune and H-1 (fig. 13) avirulent bacillus immune guinea pigs as compared with the control (fig. 11). Retardation was not evident with the small immunizing dose (fig. 14) in this animal.

In addition, the autopsy findings three to four months after virulent infection disclosed in the immunized animals either an absence or less striking evidences of organic tuberculosis than in their respective controls. The organic findings depended upon the size of immunizing injection and the total infecting dose.

In order to determine whether this same immune effect could be produced by subcutaneous injection with avirulent bacilli, a series of 4 to 6 guinea pigs for each test were treated in the following manner: subcutaneous injection of 1 mgm. (in 1 cc.) of fine suspension of (1) viable avirulent human tubercle bacilli (Human avirulent); (2) heat-killed (boiling-water-bath thirty minutes) nonviable avirulent human tubercle bacilli; (3) heat-killed nonviable virulent human tubercle bacilli (H7): and subcutaneous injection of 0.001 mgm. (in 1 cc.) of (4) viable avirulent tubercle bacilli; (5) heat-killed, nonviable avirulent tubercle bacilli; and (6) viable virulent (H7) tubercle bacilli; and (7) heat-killed, nonviable virulent tubercle bacilli. After five weeks all of the foregoing previously injected guinea pigs and 8 normal control guinea pigs were given staggered single intracutaneous injections of graded amounts 0.2 cc. of from 10^{-2} to 10^{-8} mgm. per cc. of virulent human tubercle bacilli (H7). The lesions were examined and recorded at weekly intervals. It was found that the previous subcutaneous injection of virulent human tubercle bacilli retarded the development of the lesions resulting from the intracutaneous injection of the same virulent human tubercle bacilli in graded amounts. Similarly, a previous subcutaneous injection of viable avirulent human tubercle bacilli also has an equal retarding influence upon the graded infection displayed in the nondevelopment of lesions at the site of the smaller injections. The effect was quantitative and depended on the quantity of the immunizing injection; with the smaller immunizing dose, the effect was less striking. With heat-killed bacilli, neither large nor small amounts, displayed immunizing properties. In some cases with large doses, there appeared to be an aggravation of the virulent infection as a result of the previous injection of the nonviable bacilli. The illustrations graphically depict the findings noted.

SUMMARY AND CONCLUSIONS

1. Viable avirulent human (Human avirulent) and avirulent bovine (BCG) tubercle bacilli produce no more local tuberculous skin lesions when intracutaneously injected than an equivalent amount of nonviable tubercle bacilli of the same strain or nonviable virulent strains of human or bovine tubercle bacilli.

2. From these studies and results recorded elsewhere, it does not appear feasible that dissociation of these avirulent strains of tubercle bacilli could ordinarily occur in the animal economy, since in the amounts generally injected they do not show evidences of multiplication but rather tend to become nonviable.

3. The intracutaneous injection of avirulent human or bovine tubercle bacilli into guinea pigs, rabbits, and dogs, produces gross tubercles in amounts as low as 0.01 mgm. in fine suspension. The lesions in the rabbits and dogs for equivalent amounts are larger than in the guinea pigs. The maximum effect is consummated in about three to four weeks and resolution, dependent on the dose, usually occurs within ten weeks.

4. The intracutaneous injection of avirulent human and bovine tubercle bacilli into the guinea pig one to three months prior to the intracutaneous injection of a single or double relatively large dose of virulent human or bovine tubercle bacilli results in a definite retardation in the development of the intracutaneous lesion produced by the virulent bacilli, which action resembles that actually found after a virulent (first) infection. Large doses (1 mgm.) of avirulent bacilli act more favorably than small amounts (0.001 mgm.), although an effect is still discernible in amounts as low as 0.01 mgm. Nonviable heat-killed (boiling-water temperature) avirulent or virulent tubercle bacilli produced no such retarding effect on the development of the disease following the infection with the viable virulent tubercle bacilli.

5. Coincident with the retardation in the development of the local virulent lesion caused by the primary avirulent bacillus injection, there also was noted a retardation in the development of organic tuberculosis in the immune guinea pig as compared with the normal control.

Valuable assistance was rendered in performing the experiments recorded in this study by Miss C. Clark and Mr. L. D. Miller.

REFERENCES

The References will be found at the end of Study V, where those of all the papers in this series are assembled.

STUDIES ON THE BEHAVIOR OF TUBERCLE BACILLI WITHIN THE BODY

V. THE EFFECT OF THE INOCULATION OF AVIRULENT TUBERCLE BACILLI ON SUBSEQUENT VIRULENT INFECTION IN ANIMALS

H. J. CORPER,[1] A. P. DAMEROW[1] AND MAURICE L. COHN[1]

In earlier reports by us (Papers I, II, III, IV, this series), it was shown that local cutaneous immunity to tuberculosis is produced by previous infections with virulent human or bovine tubercle bacilli; that local cutaneous immunity to virulent human and bovine tubercle bacilli in the guinea pig is likewise produced by previous injections with avirulent human and bovine tubercle bacilli; that avirulent human and bovine tubercle bacilli produce in animals (the guinea pig, the rabbit, and the dog) only nonprogressive healing lesions and that the bacilli in these lesions do not multiply but become nonviable, making it unlikely that dissociation—if this exists for human or bovine tubercle bacilli—can occur; and that the bacilli injected intracutaneously do not disseminate from the site of inoculation as readily in the immune as compared with the normal, nonimmune animal. In all these studies by us, it was noted in previously immunized animals, subsequently infected with virulent human or bovine tubercle bacilli, that there was a decided retardation or even absence of internal organic tuberculosis resulting from the virulent infection as compared with normal controls similarly infected. This was also noted by Krause, who stated as a result of his studies (25) on the guinea pig, that "the leading impression which one derives from the outcome of this experiment was one of tremendously enhanced respect and admiration of the efficiency of tuberculoimmunity as an agent of defense." Within recent years, Birkhaug (31) in an excellent study showed that parenterally BCG-vaccinated guinea pigs are relatively twice as refractory to progressive tuberculosis following virulent superinfection as the nonvaccinated animals, and that oral administration of BCG in newly born guinea pigs fails to arouse any increased resistance to virulent superinfection. Acknowledging the existence of a relative immunity

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produced by previous injections of viable virulent and avirulent human and bovine tubercle bacilli, there still remains a number of questions which merit further consideration. With reference to the rabbit, Lurie (32) noted definite evidences of immunity in this animal. No reliable observations to our knowledge are available on the production of artificial immunity of the dog. Further, although our earlier intracutaneous tests showed merit to the contention of Koch and later of Krause that the presence of tubercle was essential to the development of satisfactory immunity, this factor, together with the length of persistence of this immunity after resolution of the tubercle and suitable conditions for the development of immunity, required greater elaboration. Since other avirulent strains of tubercle bacilli besides BCG had been given little consideration by others from a specificity standpoint, and since our previous work had been concerned with avirulent human strains as well as BCG, it seemed important to attempt to determine whether such specificity assumes immune biological significance. It is well known that the guinea pig is susceptible to both virulent human and bovine tubercle bacilli, which makes it likely that cross immunization may play a part in the protection offered by BCG vaccination against virulent human infection in these animals. With the striking biological differences that exist between these two strains, it would appear likely that immunological infection differences might exist.

For purposes of evaluation and elaboration upon our experimental skin findings, it also appeared pertinent that other routes of infection besides cutaneous be tried. To determine the effect not only on the local tuberculosis but as well on the tendency to metastatic organic dissemination, guinea pigs were infected intratracheally, subcutaneously, and intravenously after immunization; rabbits subcutaneously, intratracheally, and intravenously; and dogs intravenously and subcutaneously.

In the first experiments recorded below, intravenous immunization was part of this study; and, since no comparative data were available to us on the results of the intravenous injection of these avirulent strains of human and bovine tubercle bacilli, the effect of such injections in guinea pigs, rabbits, and dogs was determined.

INTRAVENOUS INJECTION OF AVIRULENT AND VIRULENT TUBERCLE BACILLI

Varying amounts of fine suspensions of avirulent human (Human avirulent and Bernstein) and bovine (BCG) tubercle bacilli were given

intravenously in amounts up to 10 mgm. This amount of these avirulent tubercle bacilli given to the dog and rabbit produced no macroscopic changes in these animals even though the animals were killed and examined at varying intervals up to six months after injection. In the guinea pig, from 1 to 5 mgm. given intravenously (ear-vein) at times produced irregular results dependent upon the individual animal. The reaction found, however, consisted of an acute splenomegaly with occasional acute lethal termination from either an acute general intoxication of the animal coincident with the splenomegaly and rarely a haemoperitoneum resulting from splenic rupture. This profound effect was noted only for BCG although the avirulent human strain in large amounts (1 to 5 mgm.) produced a transient diffuse acute splenomegaly which, in most cases, resolved within four to eight weeks without causing any typical tuberculous findings in the animal or resulting in a progressive tuberculosis in them. This is a strikingly different picture from that seen at postmortem when much smaller amounts of virulent bovine or human tubercle bacilli are injected intravenously into guinea pigs (5). The question of purity of avirulent strains has been frequently raised and, since it is conceivable that some of the results obtained with avirulent strains in which virulent bacilli have been recovered may have been due to error rather than to the actual transmutation or dissociation of the bacilli, such as might readily occur in mixing strains (in point, the Lübeck disaster was an example of such an error in confusing cultures) or to uncontrolled animal recovery from an animal susceptible to spontaneous tuberculosis. When once a mixture of virulent human or bovine bacilli with avirulent human or bovine bacilli occurs, their separation or differentiation by means of culture is unlikely and only biological animal separation is possible, but this method again introduces complications in verifying results. However, it may be questioned whether a small number of virulent bacilli can exert their disease-producing effect in a susceptible animal in the presence of a large number of avirulent bacilli. For the purpose of determining this, a series of guinea pigs (at least four for each test) were given intravenous injections of 0.000,001 mgm. of virulent human tubercle bacilli (H7), another series were given 1 mgm. (in fine suspension) of avirulent human tubercle bacilli, and a third series were given both of the foregoing mixed before injection. The guinea pigs were killed and examined seven weeks after the intravenous (ear-vein) injection and, while no gross tuberculous changes were found in the guinea pigs that had received the 1 mgm. of avirulent human

tubercle bacilli alone, both of the other two sets of guinea pigs showed a generalized disease of about equal amount involving the lungs, liver, and spleen as well as tributary glands. The same results occurred when as small an amount as 0.000,000,01 mgm. of virulent bacilli were mixed with the 1 mgm. of avirulent bacilli although the tuberculosis was slightly less extensive. This is a remarkable demonstration of the avirulence of this human strain in that the mixture contained only 0.000,001 per cent of the virulent bacilli, and yet the intravenous infection pursued its course. It also serves as a good control for immune tests to be recorded later in this paper.

EFFECT OF INJECTIONS OF AVIRULENT TUBERCLE BACILLI (HEAT-KILLED), AND OTHER ACID-FAST BACILLI ON THE COURSE OF SUBSEQUENT INFECTION WITH VIRULENT TUBERCLE BACILLI

After exhaustive tests with various species of animals and the striking similarity within the reason of proper interpretation, it was concluded that the guinea pig under carefully controlled tests was the most suitable animal for immune tests in tuberculosis, not only because of the size and ease of handling of these animals in large numbers but also because of the large latitude offered by their susceptibility to virulent human and bovine tubercle bacilli. With properly graded infection and care to avoid forced experimental conditions, the general immune phenomenon in this animal paralleled that seen in other animals, as well as in man, in so far as it has been possible to make these parallelisms. The studies in man will be recorded later. It would be impossible to give detailed results of all the findings noted in the extensive series of guinea pigs used for testing the immune reactions following various primary treatments before virulent infection, so that the results of some of the pertinent experiments have been tabulated and, with illustrations, will suffice to depict the findings and conclusions. In all tests at least 4 to 6 guinea pigs were used to determine each point regarding a definite effect, both of the immunizing agent and the graded infection, and usually experiments were repeated at least once. Accordingly, guinea pigs were treated with varying amounts of avirulent human tubercle bacilli, with heat-killed virulent and avirulent human and bovine tubercle bacilli, and also with acid-fast saprophytes, grass bacilli and others. The routes of administration used were by gastrointestinal tube, by intracutaneous injection, by subcutaneous injection, and by intravenous injection. The animals were then infected after definite intervals of time with virulent

bacilli in graded amounts, intracutaneously or subcutaneously, intratracheally and intravenously. Overwhelming infections with virulent bacilli were avoided because of the obvious futility of protecting against such infection in a highly susceptible or even relatively resistant animal.

The results of these studies in the guinea pig indicated a definite quantitative protective influence of appropriate previous injections of avirulent tubercle bacilli administered either intracutaneously, subcutaneously, or intravenously on subsequent infection with virulent human and bovine tubercle bacilli, on the local intracutaneous lesion and local lymph nodes and metastatic internal disease. In equal amounts, heat-killed (at boiling-water temperature for 30 minutes) tubercle bacilli exerted no appreciable influence. Likewise, alimentary administration of live or dead avirulent bacilli in large amounts resulted in no appreciable protection against subsequent virulent infection. From a quantitative standpoint, the findings indicated that in the guinea pig the immune effect becomes discernible particularly at the point where the avirulent bacilli begin to produce macroscopic tubercles at the site of intracutaneous injection, that is, above 0.001 mgm.

Another experiment which yielded rather unexpected results was the effect of immunization on the intravenous infection in the guinea pig. This was rather surprising, especially in view of the fact that early in the course of this study it was conceived that the immune reaction was concerned primarily in retarding spread from a local site of infection. Yet, in spite of the general deposition in the internal organs following the intravenous injection of the virulent tubercle bacilli in small amounts (in 10^{-6} mgm. and 10^{-8} mgm.), the guinea pigs that had been given subcutaneous injections of large amounts of viable avirulent human or bovine (BCG) tubercle bacilli three months prior to the virulent infection showed decidedly less tuberculosis in the lungs, liver, and spleen than the control guinea pigs. (See illustration.)

In earlier experiments on infecting dogs with virulent human and bovine tubercle bacilli, it was found that this animal was relatively quite resistant to subcutaneous infection with these tubercle bacilli, and for this reason and the fact previously noted of the tendency for even avirulent bacilli in relatively small amounts to produce marked local tissue changes, the mode of local infection did not prove entirely satisfactory for testing immune results although differences were evident with large doses in the dog. The method of choice for infecting the dog was the intravenous method (33), and yet this proved not wholly satisfactory

PLATE 1

Fig. 1. The spleens of guinea pigs seven weeks after an intravenous injection of (1) 1 mgm. avirulent human tubercle bacilli; (2) 1 mgm. avirulent human tubercle bacilli (Human avirulent) and 0.000,001 mgm. of virulent human tubercle bacilli (H7); and (3) 0.000,001 mgm. of virulent human tubercle bacilli. Note the normal size of the spleen of the guinea pig given the avirulent bacilli alone (1) and the tuberculous involvement of (2) and (3). The spleens are an index of the general tuberculous findings, (2) and (3) reflect the tuberculous changes in the lungs and liver. Although slightly less involvement occurred when 0.000,000,01 mgm. of H7 was given with and without the avirulent bacilli, the findings were about the same, indicating that the coincident large injection of avirulent bacilli did not affect the progress of the disease resulting from the intravenous injection of small numbers of virulent bacilli in the guinea pig.

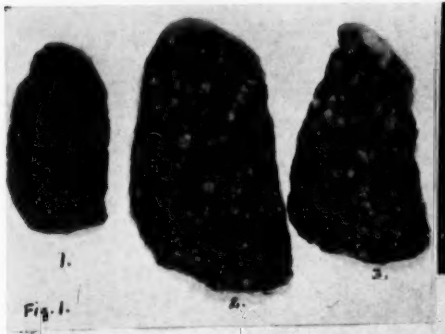
Fig. 2. The spleens and local inguinal (upper) and iliac (lower) lymph nodes in guinea pigs two months after infection subcutaneously with 0.000,01 mgm. of virulent human tubercle bacilli (H7). These guinea pigs received the following intravenous injections one to two months prior to virulent infection: (1) 1 mgm. avirulent human tubercle bacilli; (2) 0.01 mgm. avirulent human tubercle bacilli; (3) 0.001 mgm. avirulent human tubercle bacilli; (4) 1 mgm. of avirulent human tubercle bacilli (Bernstein); (5) 1 mgm. BCG; (6) 1 mgm. acid-fast grass bacilli; (7) 1 mgm. heat-killed avirulent human tubercle bacilli (Human avirulent); (8) 1 mgm. heat-killed virulent human tubercle bacilli (H7); (9) control. Note the local tributary lymph-node development dependent on the immunizing dose in (1), (2), and (3), and the lack of effect of acid-fast grass bacilli (6) and heat-killed tubercle bacilli (7) and (8). The same findings were noted when infection occurred six months after the primary injection of the same materials.

Fig. 3. Spleens of guinea pigs two months after intracutaneous infection with 0.002 mgm. of virulent bovine tubercle bacilli. (1) was obtained from an animal given 1 mgm. of avirulent human tubercle bacilli intravenously one month before infection; (2) was given 1 mgm. BCG intravenously one month before infection; and (3) was the control animal.

Fig. 4. Spleens and local tributary lymph nodes from guinea pigs three months after subcutaneous infection with 0.000,01 mgm. of virulent bovine tubercle bacilli. (1) and (2) were obtained from animals given 1 mgm. of avirulent human tubercle bacilli intravenously one month prior to infection. (3) and (4) were from control animals.

Fig. 5. Lungs of guinea pigs ten weeks after the subcutaneous infection with 0.000,1 mgm. of virulent human tubercle bacilli. (1) was from the control animal; (2) was from an animal which received an intravenous injection of 1 mgm. of avirulent human tubercle bacilli six weeks prior to virulent infection; (3) was similar to (2) but received 5 mgm. of avirulent human tubercle bacilli intravenously. Note the pulmonary tuberculosis and marked tracheobronchial glandular hypertrophy in (1) and its absence in the immune animals (2) and (3). The general involvement in these animals corresponded to the pulmonary involvement.

Fig. 6. The effect of subcutaneous immunization with avirulent human tubercle bacilli (10 mgm. three months prior to infection) upon intravenous infection with 0.000,001 mgm. of virulent human tubercle bacilli. The guinea pigs were examined five weeks after infection in this series. The lung, liver, and spleen on the left (I) were from the immune guinea pig, while those on the right (C) were from the control nonimmune guinea pig. BCG gave similar results in the guinea pig.



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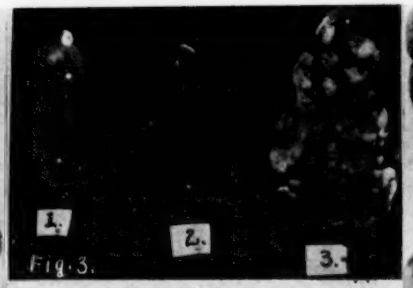

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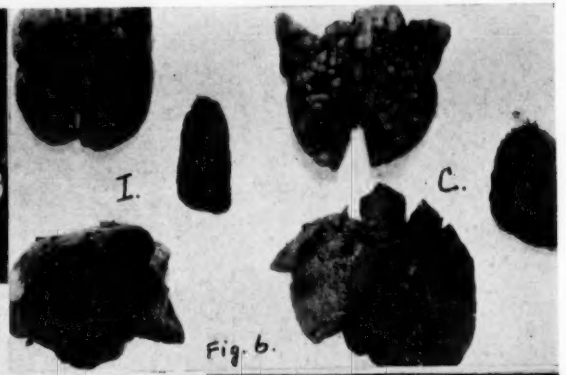

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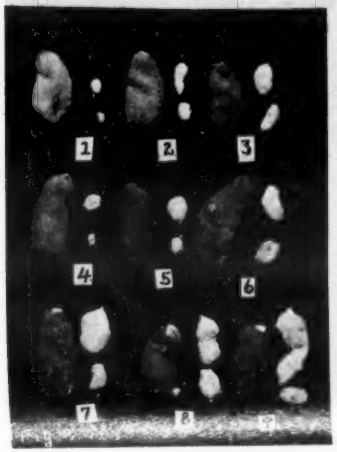

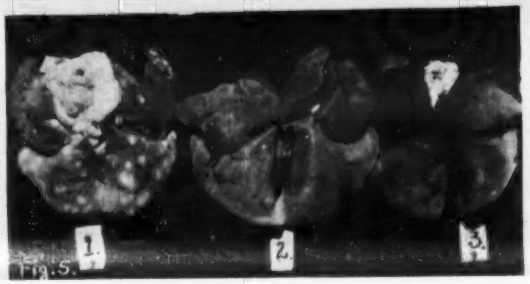

Fig. 5.

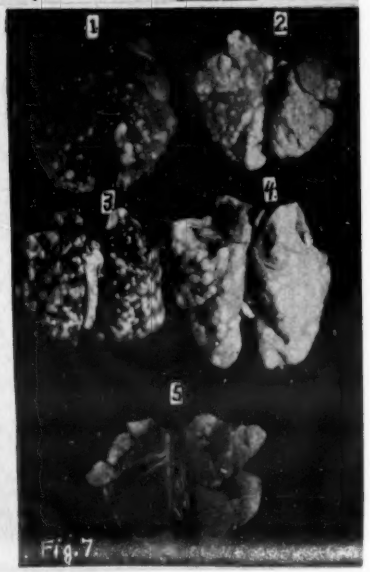

Fig. 7.

PLATE 1

Fig. 7. The effect of previous (47 days prior to virulent infection) intravenous injections of viable and nonviable avirulent human and bovine tubercle bacilli (BCG) on the development of pulmonary tuberculosis following subcutaneous infection with 1 mgm. of virulent bovine tubercle bacilli. The results are shown 100 days after virulent infection. (1) is the control animal; (2) is the animal previously treated with heat-killed (30 minutes at boiling-water temperature) avirulent human tubercle bacilli; (3) is the animal previously treated with viable avirulent human tubercle bacilli; (4) nonviable (heat-killed) BCG previously to virulent infection; and (5) viable BCG previously. Note protection in the rabbit against progression of disease produced by virulent bovine tubercle bacilli when previously immunized with viable avirulent bovine tubercle bacilli (BCG).

in view of the fact that there was only a narrow margin between the lethal and the infecting dose. In addition, the factor of natural resistance made individual findings at times quite variable. However, the dog being susceptible to both the virulent human and bovine tubercle bacilli, several series of animals were tested for their artificial immune reaction developed after the injection of viable and nonviable (boiling-water-bath for 30 minutes) avirulent human (Human avirulent) and bovine (BCG) tubercle bacilli given intravenously or intracutaneously.

The findings indicated that an intravenous injection of viable avirulent human or bovine (BCG) tubercle bacilli exerts a retarding influence on the development of organic tuberculous changes resulting from the intravenous injection of appropriate amounts of virulent human tubercle bacilli, while nonviable heat-killed bacilli in equal amounts, as in the guinea pigs, do not possess the property of producing this retarding effect in the dog.

Unlike the guinea pig and dog, which are about equally susceptible to virulent human and bovine tubercle bacilli, although slightly more so to the latter, the rabbit shows quite a striking relative insusceptibility to viable virulent human tubercle bacilli as compared with the average strain of viable virulent bovine tubercle bacilli, as was first pointed out by Theobald Smith, who used this animal for the biological differentiation of the strains. It was for this reason that it appeared to us that this pointed to a biological specificity which also might be evident from an immunological standpoint when using the avirulent human and bovine tubercle bacilli to protect against virulent human or bovine infections. Calmette hinted at such specificity but never reported on the use of a human avirulent strain of tubercle bacilli for immunological purposes (34). The literature contains rather uncertain results regarding the value of immunization with virulent human tubercle bacilli in rabbits, partly because the dosage for immunizing was not graded but excessive, and

partly because the criterion of duration of life was variable and the mode of virulent bovine infection also was conducive to irregular results. It was for this reason that a more careful and graded control test in rabbits appeared desirable. In the rabbit, the best results were obtained by intravenous immunization and subcutaneous infection, which showed the retarding influence of previous avirulent tubercle bacilli injection on the subsequent internal dissemination of the disease, as well as the effect on the local site of inoculation.

The results of such experiments indicated an apparent specificity of the avirulent bovine tubercle bacilli for retarding the local development of disease at the site of inoculation and retarding the dissemination of the disease in the internal organs following the subsequent subcutaneous injection of virulent bovine tubercle bacilli. Heat-killed (heated at boiling-water temperature for 30 minutes) avirulent human or bovine tubercle bacilli in equal amounts did not show this same effect while viable avirulent human tubercle bacilli only showed, in contrast, a slightly retarding effect.

This suggests that there is an immunological specificity which is worthy of consideration, especially since the factor of dosage plays an important part in relative lesion formation with avirulent tubercle bacilli. Although it cannot be concluded definitely that specificity against infection exists in an animal susceptible to virulent human tubercle bacilli and relatively insusceptible to bovine tubercle bacilli, it should nevertheless be borne in mind and an attempt made to verify the reverse of these findings by immunizing a species susceptible to virulent human tubercle bacilli and relatively insusceptible to virulent bovine tubercle bacilli, as is man. We are at present studying the rat for such possible differences. It also must be remembered that these differences are relative rather than absolute and that excessive doses of tubercle bacilli and extraneous infections may at times elicit protective nonspecific responses.

SUMMARY AND CONCLUSIONS

1. The question of a mixed avirulent strain of human or bovine tubercle bacilli (BCG) is discussed. It was shown that when suspensions of virulent tubercle bacilli are artificially mixed with suspensions of avirulent tubercle bacilli so that the relative amounts are in the proportion of one to ten million respectively, the virulent bacilli can be detected by intravenous injection into guinea pigs.

2. The results of the intravenous injection of avirulent human and

bovine tubercle bacilli are presented. Relatively large amounts (5 mgm.) of fine suspension result in an acute splenomegaly in this animal which usually resolves without leaving definite gross tuberculous changes. This amount of avirulent tubercle bacilli given intravenously to the dog or rabbit produced no evident gross changes in the organs of these animals.

3. In guinea pigs appropriate previous injections with avirulent human or bovine tubercle bacilli given intracutaneously, subcutaneously, or intravenously, retard the development of subsequent infections with virulent human or bovine tubercle bacilli in these animals. The effect is evident in the local intracutaneous lesions, the tributary lymph nodes, and in the metastatic organic disease developed.

4. In equal amounts, heat-killed avirulent and virulent tubercle bacilli exerted no such effect. Likewise, enteral administration of viable or nonviable avirulent tubercle bacilli in relatively large amounts was without effect upon subsequent virulent infections in guinea pigs.

5. The immune effect occurs definitely at the point where macroscopic tubercles are produced by the intracutaneous injection of avirulent tubercle bacilli in the guinea pig.

6. Notwithstanding the fact that dissemination of virulent tubercle bacilli from the site of local intracutaneous inoculation is retarded in immune as compared with normal guinea pigs, as demonstrated by Krause and verified by us, and intravenous injection causes a widespread organic dissemination of the virulent bacilli, there was noted a definite retardation in the development of the organic disease in immune guinea pigs intravenously infected as compared with control normal guinea pigs.

7. Although the dog is not entirely satisfactory for immunity tests, definite evidences of protection against virulent infections conferred by previous injections of viable avirulent tubercle bacilli are evident. Nonviable avirulent tubercle bacilli produced no such evident protection.

8. Infection experiments with virulent bovine tubercle bacilli in the rabbit suggest a definite strain (bovine) specificity for avirulent (BCG) and virulent bovine tubercle bacilli versus avirulent human tubercle bacilli. Heat-killed avirulent tubercle bacilli in like amounts exerted no retarding effect on virulent bovine infections.

We are grateful to Miss C. Clark, Mr. L. D. Miller, and Mr. Ray Stoner for assisting with the technical phases of this study.

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THE EFFECT OF IRRADIATED MILK ON EXPERIMENTAL INHALATION TUBERCULOSIS IN GUINEA PIGS[1]

DAVID F. LOEWEN AND WM. H. OATWAY, JR.[2]

INTRODUCTION

Milk has been used as a basic part of the daily diet of tuberculosis patients for centuries. The benefits to be derived from it rest largely upon empirical concepts. It is generally believed that milk is important in tuberculosis because it is a well-balanced food and not because of any specific accessory food substances.

Natural milk contains from 1400 to 2800 International units of vitamin A, and from 5 to 12 Steenbock units, or 13 to 32 International units, of vitamin D per quart. The vitamin-A content is not easily altered but Steenbock (1) and his associates in 1925 showed that by exposing milk to ultraviolet light the vitamin-D content was increased about tenfold without altering the taste or other properties. The following year Kramer (2) showed that such milk was effective in preventing rickets in infants. In 1927 irradiated dry milk became available commercially largely through the efforts of Supplee (3) and five years later irradiated fluid milk was put on the market. Milk modified by irradiation is at present readily available in many cities and its use is fast increasing.

Fish-liver oils, viosterol and other vitamin-containing drugs are being used or are being recommended for use in tuberculosis. The reported results have not been conclusive enough to make their use a routine measure nor have they been so unfavorable as to lead to their general abandonment. The vitamins, especially vitamin D, still finds enough favor among phthisiotherapists to lead one to believe that irradiated milk, as the source of this vitamin, will inevitably be used in the treat-

[1] This study was made possible through a grant from the Wisconsin Alumni Research Foundation and the coöperation of the Sheffield Farms Company, which furnished the milk. It was suggested by Dr. Charles E. North, Sanitary Engineer, who enlisted the aid of the above-mentioned organization.

[2] Fellows of the Trudeau Foundation, Saranac Lake, New York.

ment of tuberculosis. It is, therefore, desirable that experimental investigation precede clinical applications to indicate, if possible, its mode of action or rationale in this disease.

Concentrated vitamin D—that is, irradiated ergosterol—has been used by quite a number of investigators, but Menschel (4) is one of the few that has reported definite changes in the symptomatology and pathology of patients with pulmonary tuberculosis following the use of this drug. He mentions regressive changes in the inflammatory process, diminution of fever, disappearance of night-sweats, elevation of leucocyte count, which later returns to normal, or a relative lymphocytosis and slowing up of the sedimentation rate. Scheurlen and A. Orlowitsch-Wolk (5), however, were not able to corroborate these findings; and Crimm, in some extensive investigations of this drug (6) (7) (8) (9) (10), was also unable to report similar findings. Kramer, *et al.* (11) failed to note any acceleration of the healing process in patients receiving large doses of viosterol for a year; while Kaminsky and Davidson (12) noticed a rise in the serum-calcium but no demonstrable increase in calcification of tubercle by X-ray. No one has so far given a satisfactory demonstration on increased calcification in the lung-fields of patients with pulmonary tuberculosis treated with the usual doses of viosterol or codliver oil. It is, therefore, believed that the therapeutic effect attributed to codliver oil is not primarily due to a calcification of tubercles as a result of its high vitamin-D content.

Calcification, in fact ossification, of primary foci of tuberculosis in human subjects is common. Calcification likewise occurs in chronic adult tuberculosis. It has been reproduced in guinea pigs by Gardner (13), and its extensive natural occurrence in cattle has long been known. Conclusive evidence is lacking to show that calcification favors rather than follows healing, but it is possible *a priori* that a calcified tubercle is less likely to break down and liberate tubercle bacilli than one that is not calcified. Jampolis and Witt (14) concluded from their results that calcification was a reparative process rather than an expression of simple tissue necrosis. Maver and Wells (15) state that tuberculous lesions in cattle show partial calcification at all stages, yet seem to progress unimpeded by this process. A tissue such as bone, rich in calcium, is quite susceptible to the tubercle bacillus and is stubborn in healing.

Maver and Wells (15) determined the calcium content of the organs of tuberculous guinea pigs on normal and on high calcium diets, and

they found that for most organs the rule could be laid down that the more tuberculosis present the greater the calcium content. The lungs, more than any other organ, showed exceptions to this rule. In fact they found that the lungs, even when extensively tuberculous, were much less likely to show a high calcium content than either the liver or spleen. Their experiments indicated that large amounts of calcium in the diet did not cause larger amounts to be deposited in tuberculous lesions than was the case in animals on a normal diet containing the ordinarily adequate amounts of calcium.

Pfannensteil (16), Kreitmair and Moll (17), Klein (18), Smith (19), Spies (20), Vanderveer (21), and others have shown that massive doses of vitamin D in the form of activated ergosterol leads to extensive calcium deposits in the arterial walls, in the renal parenchyma, stomach walls, heart muscle, liver and lungs. Tissue degeneration, due to a toxic process, is considered to precede this calcification, but Ham and Lewis (22) have found the calcium deposits to occur within 24 hours after a large dose in rats and believe it to be a precipitation due to disturbed calcium metabolism. It has further been shown by Spies (23) (24) and by Theiss (25) that varying degrees of calcification are produced in necrotic tubercles in both guinea pigs and rabbits fed large doses of irradiated ergosterol. Simonnet and Tancret (26) determined the calcium content of lungs and found 0.10 to 0.15 per cent calcium in the dried lung of normal animals, and 0.42 to 2.06 per cent in normal animals treated with irradiated ergosterol, while in untreated tuberculous animals the calcium percentages were 0.16 to 0.68 per cent and in tuberculous animals treated with irradiated ergosterol, 1.19 to 9.68 per cent.

These observations seem to indicate that there is a direct relationship between the calcium content of the lungs and the vitamin-D intake. This was most noticeable in the tuberculous animals and might conceivably have been even more striking if the animals had been on a high calcium diet.

OBJECTIVES

It was planned to demonstrate in the present studies the possible effects of irradiated milk on the course of tuberculosis in the experimental animal. A simulation of the usual human infection was attempted. The guinea pig was used because it is susceptible to infection by the human type of tubercle bacillus. The animals were infected by

spray-inhalation because the respiratory tract is a natural portal of entry for the tubercle bacillus and because this method yields a higher percentage of pulmonary disease than the unnatural modes of infection.

The first 8 groups (series A) were unsensitized, and the disease in the infected animals was therefore of the primary infection (or childhood) type. The disease was relatively acute, virulent and progressive. The second 2 groups (series B) were sensitized before infection, and hence the disease was of the reinfection (or adult) type. It was a sluggish, resisted and regressive process.

It was presumed that these two types of tuberculosis presented a suitable contrast for a therapeutic trial.

The experimental methods, the preparation of diets, and the large bulk of data which necessarily resulted from analysis of the results are presented in almost abstract form. Any details of interest can be obtained from the authors.

Such an experimental study requires the control of all factors likely to have a bearing upon the health of the animals. There must be control groups for the most important vitamins of the milk, A and D; intercurrent infections must be guarded against; the infecting dose of tubercle bacilli must be as nearly uniform as possible; and the living conditions must be suitable and uniform. The nature of the problem makes it obvious that worthwhile conclusions can come only from large series of comparable, carefully studied animals. Finally, the marked variations in the pathology of tuberculosis, both in animals and human-beings, make it imperative that due caution be used in applying the findings derived from animal experimentation to human disease.

EXPERIMENTAL PROCEDURE

Series A: Unsensitized Animals

Four hundred animals were divided into 8 groups of 50 each, in such a manner as to obtain uniformity in distribution of sex, weight and color.

The animals were housed in a heated building with an average temperature of 60°F. Sunlight was not allowed to touch the animals. A specially constructed feeding trough was exclusive for each cage and was scoured daily. A group of 50 animals comprised a unit which consisted of 10 cages with 5 animals in each. Animals suspected of intercurrent infections were isolated in separate cages.

All animals were observed for two weeks before the inoculation. All were found to be skin-test negative to the usual intracutaneous 0.1 cc. of 2 per cent

Old Tuberculin (OT) after 48 hours. Criteria for the degree of reaction were those of the National Tuberculosis Association.

A brief record of the dietary ingredients is presented in table 1. There were 2 uninfected and 6 infected groups of 50 animals each. One group of each type—the untreated groups—received only a stock diet. One group of each type received in addition to the stock diet irradiated cream and skim-milk powder.

The irradiated milk was supplied by the Sheffield Farms Company of New York City. It was processed by them as follows: The raw grade A milk was run through a standard "Creamery Package Irradiator," where it was exposed to the ultraviolet light for two and one-half seconds. After irradiation the milk was pasteurized and run through a separator, and the 40 per cent cream which came from the separator was run over a surface cooler and immediately bottled. The cooler brought the temperature down to 42°–44°F. Seven quarts of this cream were packed in ice and sent each week to the Trudeau Laboratory, where they were stored in a cooler until used. Four potency tests, one in the laboratories of New York University, two at the University of Wisconsin and the fourth in our own laboratory, showed that the milk contained in excess of 50 Steenbock (or 135 International units) of vitamin D per quart. The cream would, therefore, have at least 500 units of vitamin D per quart.

The cream was used because of the necessity of limiting the amount of liquid in the diet of guinea pigs and because vitamin D is practically all contained in the butterfat. Since there is a limit to which milk can be irradiated without altering its taste and other properties, the vitamin dosage was largely dependent upon the amount of fat and fluid that would be tolerated by the guinea pig. It was planned to feed each animal 5 Steenbock units of vitamin D per day. The amount of cream fed during the first 51 days was 4.7 cc.

The quantity was then doubled to 9.4 cc., because an analysis showed the amount of vitamin D to be below expectations. The diet ingredients of groups 5, 6 and 7 were simultaneously readjusted to compensate. The change is indicated in the table by the two sets of figures,—the amounts fed for the first 51 days and for the 58 days thereafter.

Skim-milk powder was added to the diets of certain groups to increase their calcium content. The amount was based on that present in the milk from which the cream was derived (one quart of 40 per cent cream for 10 quarts of milk). The pigs would not tolerate such an amount. When it was reduced to 2.5 gm. per animal, it was well taken, as was the full dose (9.4 cc.) of double irradiated cream. The skim-milk powder was obtained from the Crowley Skim Milk Company, Albany, New York. It contained 1.31 per cent by weight of calcium. The amount given equals 4.6 to 5.0 gm. per day for a 150-lb. man.

The stock diet consisted of carrots, oats and hay. The carrots were specially

TABLE 1

Group diets

GROUP	STATE OF INFECTION	FOODS IN DIET	AMOUNT PER ANIMAL PER DAY	PRINCIPLES IN FOODS	AMOUNT PER ANIMAL PER DAY	CONTROL AND CONTRAST GROUPS
1	Uninfected	Irradiated cream	4.7–9.4 cc.	Fat (40%) Vitamin A Vitamin D	1.88–3.76 cc. 100–200 units 3.2–6.4 units	8 2, 5, 6
		Skim milk powder Stock diet	2.4 gm.	Calcium (0.071%) Calcium (1.31%)	0.03474–0.03804 gm.	7
2	Uninfected	Stock diet		No excess fat, Ca or Vit. D.		3, 1
3	Infected	Stock diet		Ibid., 2		2 and all infected
4	Infected	Food pellets Stock diet	*Ad. lib.*	Excess starch, protein, Vit. D, and minerals		3
5	Infected	Carotene In cotton seed oil Skim-milk powder Stock diet	0.033–0.066 gm. 1.88–3.76 cc. 2.4 gm.	Vit. A (as in cream) Fat (as in cream) Calcium (1.31%)	100–200 units 1.88–3.76 cc. 0.03143 gm.	1, 7 8
6	Infected	Viosterol (250 D) in Cottonseed oil Skim-milk powder Stock diet	0.0015 gm. 1.88–3.76 cc. 2.4 gm.	Vit. D (as in cream) Fat (as in cream) Calcium (1.31%)	5 units 1.88–3.76 cc. 0.03143 gm.	1, 7, 8, 5

7	Infected	Nonirradiated cream	4.7–9.4 cc.	Fat (40%) Vit. A Vit. D, Calc. (0.057%)	1.88–3.76 cc. 100–200 units Unimportant	1, 8, 6
		Skim-milk powder Stock diet	2.4 gm.	Calcium (1.31%)	0.03411–0.03679 gm.	
8	Infected	Irradiated cream	4.7–9.4 cc.	Fat (40%) Vit. A Vit. D Calcium (0.071%)	1.88–3.76 cc. 100–200 units 3.2–6.4 units	All groups
		Skim-milk powder Stock diet	2.4 gm.	Calcium (1.31%)	0.03474–0.03804 gm.	

prepared to act as a body for the cream or oil in the diets. The treated groups received 60 gm. and the untreated 80 gm. Hay and oats were not fed until late in the day, when the special diets were consumed and were then given ad lib.

The other four groups received stock diet, plus the added factors noted.

The rabbit-food pellets, which were obtained from the Pratt Food Company, Buffalo, New York, were fed to group 4 to determine the comparative effect of a commercial preparation containing vitamins and minerals. They are stated to contain dried buttermilk, rolled oats, linseed-oil meal, gluten meal, cornmeal, molasses, alfalfa meal, ground wheat, ground barley, codliver oil, wheat middlings, wheat bran, oats, bone meal, calcium carbonate 3/4, calcium phosphate 1/4, iodized salt 1/2 of 1 per cent.

Irradiated ergosterol was fed to provide a comparison for irradiated cream. The amount (5 units per day per animal) approximated the average of the vitamin D in the cream for the entire 109 days. It was in the form of viosterol 250 D, a product of Parke, Davis and Company.

Carotene was used to provide an excess of vitamin A comparable to that of the cream. This amount is small compared with that present in the stock diet. The drug was in the form of "Primatene" (# 1015 Carotene) prepared by the S. M. A. Corporation, Cleveland, Ohio, and kindly supplied by their Director of Research, Dr. A. F. D. Germann. It is stated to contain 3,000 ADMA units per gram.

Method of Inoculation

The spray-inhalation procedure produces no trauma incidental to infection, and a higher degree of pulmonary disease is obtainable than by infection through other portals.

The technique of infection has been previously described (27), (28). The so-called "S" variant culture of the dissociated human tubercle bacillus, H37, known to be virulent after tests previously made, was used (29). A suspension of a thirteen-day-old culture was prepared, so that a uniform smear showed an average of 0.9 bacilli per oil-immersion field (40 fields counted). A vaporizer was used which produced a fine spray. The bulb was compressed five times and the nose of the animal held in a glass tube opposite the nozzle of the spray for five seconds after the last compression. The object was to produce a slowly developing disease in the lungs with few bacilli.

Tuberculin Tests

One month after inoculation of the 300 infected animals in series A, the tuberculin skin tests were repeated. Animals which failed to react were tested again in two weeks. A third test was made eight weeks after inoculation. The results of the tests were later compared with the gross and microscopic findings.

The necrotic areas in the four-plus reactions were observed at 8, 10, 13, 16 and 20 days after the tests to determine any differences in the rate of healing. No difference was noted.

Weight Curves

The animals were weighed on the same day each week and the average weights of animals in each group were plotted as a curve. The average of the uninfected groups (1 and 2) included all animals, while those of the infected groups included only those animals found to be tuberculous at necropsy.

TABLE 2

Mortality of infected groups

GROUP	DIED		TUBERCULOSIS			COMBINED CAUSES		NONTUBERCULOUS		KILLED	
	Number in Group	Per cent in Group	Number	Per cent of "Died"	Per cent of Group	Number	Per cent	Number	Per cent	Number	Per cent
3	14	28	12	85.7	24	2	14.3	0		36	72
4	17	34	13	76.6	26	3	17.6	1	5.8	33	66
5	39	78	10	25.6	20	8	20.5	21	53.9	11	22
6	34	68	8	23.6	16	4	11.8	22	65.6	16	32
7	24	48	16	66.6	32	3	12.6	5	20.8	26	52
8	29	58	13	44.8	26	11	37.9	5	17.3	21	42
Totals and averages.....	157	52.3	72	45.8		31	19.8	54	34.4	143	

Tuberculosis: At least 7+ disease; no septicaemia or other infection.

Combination: More than 7+ tuberculosis (or pericardial effusion) *and* secondary infection.

Nontuberculous: Known cause of death (septicaemia, etc.), or cause unknown with slight tuberculosis.

Percentage of Calcium in the Lungs

The lungs of 150 animals that died and of 154 animals that were killed were analyzed for their calcium content.

The lungs of animals that died were prepared as follows: the thorax was opened and the trachea, lungs and heart removed intact. The lungs were washed, dissected free, trimmed of their protruding bronchi, blotted dry, and sectioned in various directions to determine the extent of the tuberculosis. They were then placed on a small nichrome wire hook, and hung in a drying oven at 100°C. for ten hours. The weight after drying was determined and the lung placed in a weighed platinum crucible and ashed in an electric furnace. The weight of the ash was determined and an analysis made as described below. The lungs of the animals in each group dying in the same week were usually analyzed together.

TABLE 3

Calcium analyses of lungs of killed animals

DAYS →	29–35					57–63					85–91					99–105				
GROUP NUMBER	Number of Analyses	Degree of Pulmonary Tuberculosis	Percentage of Calcium			Number of Analyses	Degree of Pulmonary Tuberculosis	Percentage of Calcium			Number of Analyses	Degree of Pulmonary Tuberculosis	Percentage of Calcium			Number of Analyses	Degree of Pulmonary Tuberculosis	Percentage of Calcium		
			Wet Lung	Dried Lung	Ash			Wet Lung	Dried Lung	Ash			Wet Lung	Dried Lung	Ash			Wet Lung	Dried Lung	Ash
1	1	0	0.0072	0.0424	0.0605	1	0	0.0071	0.0440	0.5793	3	0	0.0082	0.0414	0.6923	5	0	0.0087	0.0432	0.6846
2	1	0	0.0077	0.0438	0.6450	1	0	0.0069	0.0414	0.6832	3	0	0.0090	0.0465	0.7831	5	0	0.0086	0.0434	0.7113
3	1	1	0.0080	0.0448	0.6204	1	2	0.0123	0.0630	0.9418	3	2.3	0.0169	0.0856	1.2749	29	2.4	0.0198	0.1034	1.5726
4	1	1	0.0079	0.0438	0.6130	1	2	0.0164	0.0817	1.1862	3	2.3	0.0169	0.0874	1.3164	27	2.5	0.0249	0.1314	1.9902
5	1	1	0.0085	0.0415	0.5688	1	1	0.0073	0.0366	0.5417	1	3	0.0106	0.0571	0.9097	5	2.6	0.0164	0.0873	1.4039
											1	0	0.0080	0.0478	0.6540					
											1	0	0.0068	0.0329	0.5099					
6	1	1	0.0071	0.0404	0.5256	1	2	0.0088	0.0497	0.6530	3	2.3	0.0106	0.0518	0.7651	5	0	0.0064	0.0563	0.6732
																5	2.2	0.0143	0.0768	1.0659
7	1	1	0.0075	0.0415	0.5526	1	2	0.0127	0.0669	0.9969	1	2	0.0140	0.0710	1.1023	1	0	0.0099	0.0399	0.6421
											1	2	0.0252	0.1311	1.4654	17	2.3	0.0128	0.0794	1.1626
											1	0	0.0078	0.0399	0.6265					
8	1	1	0.0084	0.0483	0.6530	1	2	0.0108	0.0564	0.8359	1	0	0.0074	0.0365	0.5832	4	0	0.0079	0.0423	0.6791
											1	2	0.0097	0.0501	0.7493	11	2	0.0133	0.0724	1.1243
											1	3	0.0096	0.0398	0.7541					

TABLE 4

Calcium analyses of lungs of animals that died

DAYS →	GROUP NUMBER	3	4	5	6	7	8
43–49	Number of Analyses			1			
	Degree of Pulmonary Tuberculosis			1			
	Percentage of Calcium in Ash			0.62			
50–56	Number of Analyses			1			
	Degree of Pulmonary Tuberculosis			2			
	Percentage of Calcium in Ash			0.77			
57–63	Number of Analyses	1	2	5	1		
	Degree of Pulmonary Tuberculosis	1	2.5	2.2	1		
	Percentage of Calcium in Ash	1.09	0.73	0.96	0.33		
64–70	Number of Analyses	1	2	11	4	4	5
	Degree of Pulmonary Tuberculosis	2	2.3	1.5	1.5	2.2	2
	Percentage of Calcium in Ash	1.41	1.73	1.52	0.68	0.94	0.72
71–77	Number of Analyses	2	3	4	9	3	2
	Degree of Pulmonary Tuberculosis	3	2.3	1.7	1.7	2.3	3
	Percentage of Calcium in Ash	1.94	1.68	1.40	1.77	1.72	1.07
78–84	Number of Analyses	4	4	5	5	8	6
	Degree of Pulmonary Tuberculosis	2.7	3	2.4	1.2	2.6	2.5
	Percentage of Calcium in Ash	2.29	1.70	1.37	0.88	1.02	0.97
85–91	Number of Analyses	1	1	3	6	3	5
	Degree of Pulmonary Tuberculosis	4	1	2	1.3	2.3	2
	Percentage of Calcium in Ash	1.08	3.39	1.25	1.12	2.48	1.21
92–98	Number of Analyses	3	3	2	2		3
	Degree of Pulmonary Tuberculosis	4	1.6	2.5			2
	Percentage of Calcium in Ash	1.30	0.98	0.98			1.07
99–105	Number of Analyses	2	1	2	2	4	6
	Degree of Pulmonary Tuberculosis	3.5	3	2	0	2.7	2.8
	Percentage of Calcium in Ash	1.34	2.17	2.00	0.62	1.03	1.39

In animals which were killed the procedure was as follows: The animals were killed by a blow at the base of the brain. The thorax was opened and a cut made in the heart to permit free bleeding. The trachea, heart and lungs were immediately removed intact, the lungs inflated to about twice the relaxed size and the trachea tied. The inflated lungs were rinsed in distilled water. After draining for 5 minutes they were dissected free as before, blotted dry, weighed, and the weight determined of tissue saved for pathological study. The lung was then put on hooks, dried as described above, incinerated and analyzed.

The calcium was determined by the method of Clark and Collip (30), modification having been made where necessary. In the case of individual lungs the ash was dissolved in one cubic centimetre of distilled water with the aid of a few drops of concentrated hydrochloric acid and transferred to a 15-cc. graduated pyrex centrifuge tube. The crucible was thoroughly rinsed with five 1-cc. portions of distilled water. Concentrated ammonium hydroxide was added, drop by drop, until the solution became just alkaline, using a drop of methyl-red as indicator. Two cubic centimetres of 4 per cent ammonium oxalate were added and then just enough acetic acid to make the solution acid. The tube was shaken and placed in the ice-box overnight. After centrifugating for 10 minutes at 1800 r.p.m., all but a small drop of the supernatant solution was siphoned off, using a capillary tube with a small tip bent upwards. The precipitate was washed in the centrifuge tube with 3 cc. of cold 2 per cent ammonium hydroxide, centrifugated for 10 minutes and the supernatant fluid siphoned off. The washing was repeated twice. Two cubic centimetres of N/10 sulphuric acid were then added, the tube placed in a boiling water bath for 1 minute, and then titrated at 70°C. with N/100 potassium permanganate. Each cubic centimetre of this latter solution is equivalent to 0.2 mgm. of calcium.

This method in our hands was accurate to within 3 per cent when a known solution containing 0.5 mgm. of calcium was incinerated in the platinum crucible and run through the above procedure, and to within 7 per cent when a solution containing 0.1 mgm. calcium was added to an aliquot portion of dissolved lung ash.

Pathology

Procedure: Animals dying during the course of the experiment were quickly autopsied. They were opened, observations were made on the extent, distribution, and type of disease, and a search was made for possible calcifications. The condition of the tracheobronchial lymph nodes was noted carefully; the heart and aorta were examined in each case; if the cause of death was not evident, heart's-blood cultures were made on pleural-fluid broth, and examined after 24 and 48 hours, and any questionable lesion was examined for tubercle bacilli or a piece of the tissue was saved for microscopic study.

Animals from each diet group were killed for gross and microscopic compara-

tive study at 7, 34, 62 and 91 days. The remaining infected animals were killed between 102 and 112 days. Sections of the lung, liver, spleen, kidney, femoral marrow, heart and aorta, tracheobronchial lymph nodes, and any other unusual structure were preserved in 10 per cent neutral formalin. Tissues were saved from all animals killed during the first twelve weeks; from 3 animals in each group during the termination; and from animals which contained no gross evidence of tuberculosis. Supravital studies of the tissues of the animals killed at one week were made for early information as to the presence of infection.

The tissue sections were allowed to remain in formalin for 24 to 48 hours and then transferred for temporary storage to 80 per cent alcohol. They were run into paraffin blocks by the usual alcohol-xylol-cedaroil-xylol method of dehydration, clearing, infiltrating and embedding. Sections were cut 5 micra in thickness and, after deceration and hydration, were stained with Harris's haematoxylin and eosin. The formalin and haematoxylin method was used because of its ability to preserve and stain calcium deposits.

Macroscopic observations: Infection was evident, as seen by supravital studies, at one week. At 2 weeks there were small, peripheral, gelatinous tubercles in the lungs. These had become typical acute primary complexes at 4 weeks (comprised of a few discrete large caseous tubercles with marked tracheobronchial lymph-node involvement). By 8 weeks the first pulmonary lesions had decreased in size but become caseous or purulent, a pulmonary spread had occurred, and the nodes were also smaller and softened. Spread to the organs had occurred and a difference in spleen size in certain groups had become noticeable. Deaths from tuberculosis began at this time. At 12 weeks the average amount of tuberculosis and the spleen size per animal had become similar in all groups.

Certain deviations from the described course occurred. There were several deaths from septicaemia or unknown cause at 2 weeks. At 7 weeks a number of animals developed tuberculous pericardial effusion, which seemed to be related to the contiguous lymph nodes which were enormously large. Some of these animals died (of cardiac failure or asphyxiation) and some were found with organized lesions later. The occurrence of these cases among the groups was irregular.

The mortality figures are listed in table 2. They are classified as to general causes of death and by group. Survival is too uncertain a criterion for comparison of lesions in virulent tuberculosis of guinea pigs. The disease becomes generalized in all. For this reason, and because of the sacrifice of animals for comparison at certain intervals, no figures concerning the average duration of life of the diet groups are included. Rather than this, other more successful methods were used.

Analysis of the causes of death and of the degree and character of tuberculous involvement, as seen both macroscopically and microscopically, was made in detail. The results will be mentioned and discussed in the summary.

A correlation of the gross pulmonary pathology with single film roentgenograms was attempted. The films were taken immediately before sacrifice of the animals. In certain cases the progress was followed by several weekly examinations before the demise.

Series B: Sensitized Animals

A second experiment was done with the intention of protecting the animals by vaccination, infecting them through the respiratory tract, and examining them for differences in amount, distribution and type of tuberculosis. Such disease should be similar to the chronic reinfection, human type and any protective value of the vitamin-D cream might be demonstrated more easily in this less acute, resisted disease. The experiment was simplified by the use of only two diet groups. Weight observations and gross pathological examinations were the only studies pursued.

Procedure

Fifty animals, distributed as in Series A, were used in each group. The first group (9) was the control series and the daily diet contained unirradiated market cream (40 per cent fat content); skim-milk powder; stock diet (carrots, oats, hay). The second group (10) was the test series and the daily diet contained irradiated cream (40 per cent fat-content); skim-milk powder; stock diet (carrots, oats, hay).

The difference in the diets is the irradiation of the cream fed to group 10. The amount of this vitamin-D factor as well as the amount per guinea pig of the cream, skim-milk powder, carrots, oats, hay were the same as that described for groups 7 and 8, series A. All animals were taking their diet portions satisfactorily for ten days before the experiment began. They were weighed at weekly intervals. All were skin-test negative at this time.

The sensitizing and infection were the same for both groups. Each animal was given 1 mgm. (dry weight) of a 30-day culture of living R1 bacilli subcutaneously in the left groin. The strain is avirulent and does not produce progressive tuberculosis. After 3 weeks the animals were skin-tested, and all reacted positively. They were then given a spray-inhalation (as in series A) with a suspension of human (H37) "S" variant containing an average of 1.0 bacillus per oil-immersion field. (In this and in later sprayings the numerical

sequence of the animals was disordered and the tasks of the technicians were "staggered" to minimize the possible effect of these factors on infection.)

Examination 4 weeks later of unsensitized controls and two animals from each diet group (by roentgenogram and necropsy) indicated that the pulmonary infection was not extensive. There were regressing, localized abscesses at the sites of vaccination and enlargement of the inguinal lymph nodes.

Six weeks after the first spraying the procedure was repeated, using a suspension containing an average of 2.0 bacilli per field. Specimens from diet and control groups, taken after a month interval, again indicated a slight infection.

The third and final spray-inhalation was given 6 weeks after the second (and 15 weeks after the sensitizing dose). A virulence test of the spray suspension on guinea pigs (intratesticular) produced extensive disease.

Twenty-one weeks after the first pulmonary infection it was seen, from sacrificed, sensitized controls, that the disease was still well controlled and slight, and that the desired infection result had been obtained. The remaining 72 animals were therefore sacrificed. The same technique was used as in Series A.

SUMMARY AND DISCUSSION

The following conclusions may be drawn from the results of experiments on unsensitized animals (series A):

1: The effect of the various diets on the general health of the animals was judged by the weight-curves. The uninfected group receiving irradiated cream maintained a higher average weight-curve than its control group. The infected group receiving irradiated cream had one of the two highest sustained averages (a commercial preparation containing supplementary mineral and vitamin substances was the other). The curve was similar to but higher than those of the carotene and viosterol groups and considerably higher than the nonirradiated cream group.

2: The administration of irradiated cream to group 8 was not followed by a decreased general mortality in comparison with its control group (3) and with groups on contrasting diets, nor did it cause a remarkable change in tuberculosis mortality. Its use was not accompanied by an increase in secondary infections, although there were slightly more than in a control group. The general mortality of group 8 was third (out of 6 groups) and its tuberculosis mortality was fourth.

Groups 3 (stock diet) and 4 (food pellets) had the lowest general mortality, but the deaths were almost entirely due to tuberculosis; the common-diet factor, perhaps responsible for the lack of secondary infections, was the absence of skim-milk powder (Ca). Groups 5 (carotene)

and 6 (viosterol) had the highest death-rate from secondary infection or unknown cause; they received cottonseed oil as a common dietary ingredient. It has been reported that cottonseed oil contains a substance called "gossypol," which is noted to be toxic for laboratory animals (31).

The animals were separated in groups of five during life and the statistics failed to show epidemic deaths among cage mates. Administration of vitamin D was not followed by any toxic manifestation nor by calcification of the tissues.

3: The average amount of disease per group and the organic distribution of the lesions were noted at various stages of the experiment. The average amount of disease in the group receiving irradiated cream was one of the three lowest. The average spleen size was among the lowest. The average amount of pulmonary tuberculosis was one of the two highest. These tendencies were all definite but small. An explanation for the limitation of disease depends on several speculative factors and cannot be held conclusive. There was no common dietary basis to link the groups which had averages similar to those of group 8.

The amount of disease was seen to be about the same microscopically as had been estimated grossly.

DeCarvalho (32) demonstrated in a small number of guinea pigs that animals infected with tuberculosis and treated with Vigantol had less tuberculosis than their controls (untreated or treated before infection). Theiss (25) found that small doses of the same preparation were more effective than large doses or none.

4: The type of disease as seen microscopically could not be said to vary with diet. Evidences of sluggish progress and of healing were present, but they did not predominate in any group or groups. The type of disease was associated with the amount of disease which would seem to indicate that its progress was determined by the infecting dose, the portal of entry, and individual resistance. The pulmonary disease in every case was that produced, primarily or secondarily, by bronchogenic spread. Lesions in other organs were the result of haematogenous spread. The splenic type of disease was frequently acute. Caseation was not prominent either grossly or microscopically. There was no relationship found between the amount of caseation and diet. It was obviously found with increasing frequency as the experiment progressed; in thin or thinning guinea pigs; and more frequently in those that died than in those that were killed.

5: Calcification could not be determined macroscopically or by X-ray with certainty. Microscopically, it occurred in tuberculous lesions in extracellular sites, but not in the tissue itself. It was not found in any location in nontuberculous animals. Its occurrence in tuberculous lesions was in the oldest (primary) caseonecrotic lesions only, and hence it was uncommon. It is quite possible that the limitation of caseation and necrosis prevented more obvious calcification. (Spies (33), Menkin (34), and others found calcifications only in the tuberculous sites which were degenerated. It is considered by Jampolis and Witt (14) to be a reparative process, and by Sweany (35) similarly to be a step between necrosis and petrifaction.)

Calcification occurred in all diet groups and to about the same extent, both in tuberculous lesions and tissue spaces, and hence diet (and excesses of vitamin D and calcium) could not be said to have an effect on its production.

Although the relatively slight necrosis may have inhibited calcium deposit, the amount of vitamin D in the milk, as well as in the Viosterol, was possibly insufficient to cause marked calcification and probably far less than that necessary to cause changes in normal tissues. This would agree with Spies (23) and with Jampolis and Witt (14), who believe that the larger doses are necessary to produce calcification, especially in nontuberculous tissues.

Calcium deposits were found in the vascular lumina and in renal tubules, but not in the tissues in the present experiment. These nontuberculous sites have been previously described (33, etc.). The findings of Regher and Walkoff (36) of the deposition of calcium in the bone, heart and kidney tissue after administration of irradiated foods cannot be substantiated. Harris and Innes (37) believe that the most favorable regimen for calcification of the tissues is the administration of calcium for a long time and enough excess vitamin D to cause a small loss of weight.

In the few nontuberculous animal studies there was no evidence of calcium deposits in any site, even on diets with increased calcium and vitamin D. This compares in general with the results of Spies and Berryhill (24) who found greater deposits in tuberculous animals which were fed viosterol than in normal animals on far larger doses. The cause is considered to be a part of the peculiar, unexplained calcium metabolism of tuberculous subjects. In this same category are the results of lung analyses.

The deposit of calcium in tuberculous animals without large increases of vitamin D in their diet would necessarily occur from blood with a normal calcium level. No blood-calcium determinations were done here, but notable increases in blood calcium have not been reported in untreated tuberculous animals or humans.

6: The results of the analysis for calcium content of the lungs of the animals that died and of those that were killed are given in tables 3 and 4. There were slightly greater variations between the amounts of tuberculosis and the amounts of calcium in the group that died, a phenomenon which was due to the more frequent presence of fluid and of caseation.

The same conclusions may be drawn from both groups:

1: Vitamins A and D, alone or together (as in irradiated cream) and in the concentrations used, did not exert any noticeable influence on the calcium content of the lungs of tuberculous or nontuberculous guinea pigs.

2: The calcium content of the lungs of nontuberculous animals is fairly constant and is uninfluenced by increased calcium in the diet.

3: The calcium content of the lungs of tuberculous animals is increased proportionately to the degree of tuberculosis of the lungs. It is independent of the amount of calcium in the diet.

The finding that the calcium content of the diet and the calcium content of tuberculous and nontuberculous lungs are independent confirms the results of Maver and Wells (15). However, they were unable to correlate the degree of tuberculosis with the calcium content of the guinea-pig lungs. This may have been because their method of infection was subcutaneous and they obtained very little pulmonary disease. Barkus (38) found an especially high calcium content in lungs of extensively tuberculous animals exposed to inhalations of marble dust. In the present experiment the calcium content was normal in uninvolved lungs of animals with visceral tuberculosis.

The lack of vitamin D effect on the calcium content of the lungs was to be expected. Previous reports have indicated that the intake must be very high to be effective. The results of Simonnet and Tanret (26) could not be confirmed. They found an increase of the calcium in the lungs of healthy rabbits and an even greater amount in the lungs of tuberculous rabbits treated with large doses of irradiated ergosterol,—conditions which may be due to the size of the dose and certainly to the animal species.

The percentage of calcium in the fresh normal lungs closely approaches the lower limits of normal for blood-calcium. When determined on the basis of desiccated lung, the figures agree with those of Maver and Wells (15) for guinea pigs (0.0485 per cent). They are much higher than Barral and Barral (39) for rabbits lungs (0.0048 per cent) but the accuracy of their method is questionable.

The calcium analyses bear out the now prevailing belief that there is no calcium demineralization in tuberculosis.

7: Calcium in the diet was not a factor in limiting the amount of tuberculosis nor in increasing the calcification of its lesions. The first result again agrees with Maver and Wells (15) who concluded that the administration of calcium had not reduced the amount of spread of tuberculosis or led to fibroplastic tissue reaction in guinea pigs. In the dosage used (in the form of skim-milk powder) calcium was not toxic. It is possible that its use was concerned with the rate of non-tuberculous deaths in the groups receiving it.

8: Vitamin A was available in the diet of all groups in such ample amounts that the statistics of the carotene group are insignificant. In the doses given, it did not change the resistance to tuberculosis, protect against secondary infection, nor cause untoward effects.

9: It was not possible to demonstrate the exact quantity or quality of pulmonary disease by roentgenogram. It was possible to diagnose the presence of disease, and the locus of the larger lesions, but lesions at autopsy could not always be seen on further inspection of the films. This could easily be due to the small size of the lesions and the very early stage of calcification. The calcium deposits were not far advanced enough to simulate the usual radioöpaque calcification.

10: The feeding methods and the number, grouping and distribution of the animals seemed satisfactory enough to minimize the effects of individual susceptibility to diet or disease.

11: The effectiveness of the spray-inhalation method in producing pulmonary tuberculosis in guinea pigs was demonstrated. Almost all animals with any disease at all had some lung lesions. It was not as effective in producing infection as a parenteral infection, but a considerably higher percentage of animals were tuberculous at autopsy than has previously been reported by insufflation. Ninety-one per cent of those sprayed had gross disease.

Ninety-seven per cent of the animals gave a positive tuberculin test in some degree. The discrepancy between the percentage with gross

disease and the percentage with positive tuberculosis is explained by two factors: the less intense reactions were associated with little or no disease, and the microscopic examination of all grossly negative animals, while absolutely confirmatory in its failure to find tubercles, often showed a slight thickening of the alveolar walls and a stimulation of the pulmonary lymph foci,—a possible appearance of resolution. The tendency of the method to produce a uniform infection is open to question. An analysis of the mortality figures suggested that the sequence of spraying may have been associated with extent of disease. This has been borne in mind when interpreting possible dietary effects.

The following comments may be made from the results of experiments on sensitized animals (series B):

1: A group of guinea pigs, receiving a diet containing irradiated cream (before and during the development of tuberculous sensitivity and repeated pulmonary reinfection), exhibited only slight differences in the macroscopic extent, distribution, or type of disease when compared with a control group receiving a diet containing unirradiated cream under similar circumstances. There was no gross evidence of calcium deposition in either group. The group receiving the irradiated cream maintained a slightly greater weekly weight average than its control group. The difference amounted to from 15 to 25 gm. per week.

Sensitization gave the guinea pigs a remarkable protection. Forty-one animals on the irradiated cream diet were grossly tuberculous, but only to the average extent of 2.0 plus (out of a possible 10 plus). Forty-five of the control cream animals were grossly tuberculous with an average of 2.5 plus disease. The lesions were most frequently confined to the lung and tracheobronchial lymph nodes; when more extensive, the spleen and rarely the liver contained lesions; and the type of disease was sluggish, fibrous and productive and caseation was very rare.

CONCLUSIONS

1. Irradiated cream was added to the diets of groups of normal and sensitized guinea pigs. These animals were then infected by the spray-inhalation method. Uninfected and control groups receiving vitamins A or D or calcium in control or comparable amounts were established. A total of 500 animals was used.

2. Under this regimen no remarkable prophylactic or therapeutic effects were found, as judged by the extent or type of the resultant

lesions and by the mortality statistics, when the pathology of the groups receiving the irradiated cream was compared with that of the control groups. Tendencies suggesting a favorable effect were noted in the groups receiving irradiated cream but they were not considered great enough to be of significance. It is possible that the tendencies might be of different importance when translated to human infection.

3. The groups receiving irradiated cream exhibited a sustained, higher average weight curve than did their control groups.

4. Calcium deposit was found during gross and microscopic examination only in the tissues (and lesions) of tuberculous animals. The calcium content of the lungs of the infected animals was greater than that of the uninfected, and was in quantitative relationship to the amount of disease present in the lungs. The occurrence of calcification and of abnormal calcium content of the lungs was independent of the vitamin or calcium intake of the animals. The administration of irradiated cream was not followed by untoward effects.

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EXPERIMENTAL TUBERCULOUS ALLERGIC SEROSITIS AND ITS RELATIONSHIP TO HUMAN POLYSEROSITIS[1,2]

TASKER HOWARD AND J. ARNOLD DEVEER

Some time ago, having encountered a number of cases of polyserositis in patients, we became interested in learning the mechanism by which this progressive fibrosis of the lining membranes of a number of serous cavities could be brought about. Some years before this it had been shown that tuberculous effusions in the serous cavities are dependent upon sensitization to tubercle bacilli or their products, in other words, that they are allergic in nature. Rist, Kindberg and Rolland (1) in 1914, had produced an immediate bloody, ascitic effusion in tuberculous guinea pigs by the intraperitoneal injection of living tubercle bacilli. Animals not previously infected, responded by the gradual development of tuberculosis without any immediate exudative reaction. Three years later Paterson (2) obtained similar results in the pleural cavity. Petroff and Stewart (3) finally proved the allergic nature of these reactions. They employed killed cultures of tubercle bacilli in order to sensitize guinea pigs. The subsequent introduction into the peritoneal cavity of 3 cc. of a 25 per cent solution of Old Tuberculin always caused a violent reaction, usually fatal, in sensitized animals. All these investigators made note of exudation into serous cavities other than the one into which the "shocking dose" of antigen had been introduced. Much further work has been recorded as to the nature and characteristics of these serous-cavity reactions in experimental tuberculosis. We shall not attempt to review this except in so far as it relates to our immediate problem.

Chronic progressive multiple serositis in man is characterized clinically by recurrent attacks of effusion into various serous cavities, and pathologically by progressive thickening of the serous membranes, which show evidence of a chronic inflammatory process. In the deeper layers is found more or less cellular infiltration of an acute or subacute type

[1] From the Departments of Medicine and Pathology of the Long Island College of Medicine, Brooklyn, New York.

[2] Read before the Long Island Clinical Society.

while the more superficial portions of the thickened membranes are composed of dense connective tissue, usually hyalinized.

It is not unlikely that this syndrome can be produced by various pathological agencies. In considering the possibilities, the question presented itself as to whether recurrent allergic reactions could give rise to such proliferative changes, and, if so, whether tuberculosis might, at times, constitute the basis of the allergic reactions. That this might be true was suggested by the fact that Concato's (4) first 2 cases (1881), Curschmann's (5) original case (1884), and one of Pick's (6) first 3 cases (1896) showed tuberculosis at autopsy. In fact many of the recorded cases of polyserositis showed some evidence of tuberculosis, although it should be stated that this lesion was specifically excluded in the reports of some cases. Furthermore the tendency to undergo calcification, particularly of the pericardium, is suggestive of a tuberculous origin.

We have therefore conducted a series of experiments designed to show (1) whether repeated allergic reactions can be evoked by the injection of tuberculin into one or another of the serous cavities of tuberculous guinea pigs; (2) whether repeated allergic reactions may result in fibroid thickening of the serous membranes of the cavity into which the tuberculin is introduced; (3) whether the serous membranes of cavities not directly exposed to the tuberculin may also become thickened by this procedure. In short, we were attempting to produce chronic multiple serositis by repeated injections of an antigen into one serous cavity of sensitized animals.

While the experiments have not resulted in the reproduction of the syndrome of polyserositis we feel that a preliminary report of our observations may be of interest.

EXUDATIVE REACTIONS

Seventy-seven guinea pigs, infected by subcutaneous inguinal inoculations with the virulent H37 strain of tubercle bacillus, were given from one to seven intraperitoneal injections of from 3 to 5 cc. of Old Tuberculin[3] in dilutions varying from 3 to 5 per cent. The injections of tuberculin were begun only after the development of a palpable inguinal lesion, usually from two to four weeks after the infecting dose, or after the animals were shown by intracutaneous test to be sensitized. Au-

[3] Old Tuberculin from the New York City Department of Health.

topsy, 24 hours after the last injection of tuberculin, revealed a large or small peritoneal effusion in 62 (80.5 per cent) of the animals, while 17 (22 per cent) showed pleural effusions as well, and, in a few cases, there appeared to be some excess fluid present in the very small pericardial sac (table 1).

The serosa of the injected cavity invariably showed more or less congestion and, on microscopic examination, exhibited, in addition, oedema and a variable degree of leucocytic infiltration, the polymorphonuclears predominating. There was always present some exudate in the form of fibrin infiltrated with leucocytes. These changes were present to some extent in all the tuberculous guinea pigs injected even when the amount of fluid exudate did not exceed the normal (0.5 cc.), in which event the animals were classified as showing no effusion. In only one

TABLE 1

Tuberculous guinea pigs autopsied 24 hours after single intraperitoneal injections or multiple (biweekly) injections of 5 cc. of from 3 to 5 per cent O.T.

STRAIN OF TUBERCLE BACILLUS	NUMBER OF ANIMALS	EFFUSION		
		Peritoneal	Pleural	Pericardial
"H37"	77	62 (80.5%)*	17 (22%)	2 (2.5%)
"R1"	10	7 (70%)	0	0

* It is possible that the proportion of animals in this series reacting with the production of ascites was modified in some degree by the time relation of the injection to previous skin tests. Other factors possibly operative were the stage of infection and the size of the animals.

instance was a frankly tuberculous productive serositis encountered, although in a few advanced cases scattered tubercles were found beneath the surface of the spleen or the liver.

When multiple injections of tuberculin were employed, an interval of two weeks was found sufficient to permit resorption of the exudate in the serous cavities and reëstablishment of a hypersensitive state as indicated by intracutaneous tests. It was our practice to kill and to autopsy one of each group of guinea pigs so treated 24 hours after each biweekly intraperitoneal dose. Almost invariably the autopsied animal showed a more or less marked peritoneal effusion and the remaining members of the group were assumed to have reacted similarly.

There was a striking tendency for the severity of the reaction to parallel the extent of the infection. This is in accord with the relation of the severity of the skin reaction to the extent of the infection, as

pointed out by Römer and Joseph (7) and substantiated by Krause (8). Early or mild infection gave rise to smaller amounts of fluid with fewer leucocytes and but little blood, while the reactions evoked in animals with widespread tuberculosis were often severe enough to kill and were characterized by the production of bloody ascitic fluid containing many pus-cells.

In 8 nontuberculous guinea pigs the injection of 5 cc. of 5 per cent O.T. into the peritoneal cavity caused no effusion.

This series of experiments served to confirm the findings of others regarding the allergic nature of effusions into serous cavities in tuberculosis, and indicated that, with suitable sublethal dosage, tuberculin could be used to elicit repeated intraperitoneal allergic reactions in tuberculous guinea pigs. No appreciable degree of fibrosis of the serous membranes could be found, however, even after as many as 7 intraperitoneal injections, as in two of the animals, and it was evident, as suggested by Doctor Edward R. Baldwin, that chronic changes would be more likely to follow prolonged treatment of animals infected with an organism of lower virulence.

Ten guinea pigs infected by subcutaneous inguinal inoculation with the R1 strain of tubercle bacillus were given one intraperitoneal dose of 3 per cent Old Tuberculin. The inflammatory reactions were, in general, much less marked than in animals infected with the H37 strain. In only three, however, was there less than 0.5 cc. of exudate, the remaining 7 animals showing from 0.5 to 4.5 cc. of serofibrinous peritoneal effusion. None of these showed an increase in fluid in the pleural or pericardial sacs (table 1).

PRODUCTIVE REACTIONS[4]

In another series 12 guinea pigs similarly infected with the R1 strain were given repeated intraperitoneal injections of Old Tuberculin at intervals of two weeks or more over a period of about six months. Five members of this group were killed and autopsied after 3, 4, 5, 6 and 7 injections, respectively, of tuberculin. We found it advisable to employ stronger doses as the treatment proceded, and therefore used 5 cc. of O.T. in dilutions increasing from 3 to 15 per cent and even, in one instance, to 20 per cent. All of these animals showed abundant peritoneal effusions varying from 3.5 to 10 cc.

[4] The word "Productive" is used in both the text and the accompanying tables to indicate diffuse fibrosis and not tubercle-formation.

N.B.: In these tables the word "Productive" is used to indicate a diffuse fibrosis and not tubercle-formation.

TABLE 2

A: Animals infected with R1 strain of tubercle bacillus and given repeated intraperitoneal injections of 5 cc. of from 3 to 15 per cent O.T. (strength of average dose 8 per cent)

NUMBER OF ANIMALS	NUMBER OF INTRAPERITONEAL INJECTIONS	INTERVAL BETWEEN LAST INJECTION AND AUTOPSY	EFFUSION			MICROSCOPIC CHANGES IN SEROUS MEMBRANES					
						Peritoneum		Pleura		Pericardium	
			Peritoneal	Pleural	Pericardial	Exudative	Productive	Exudative	Productive	Exudative	Productive
667	3	24 hrs. (killed)	3.5 cc.	0	0	slight	0	slight	0	not examined	
670	4	24 hrs. (killed)	8.5 cc.	0	0	slight	0	0	0	slight	slight
679	5	48 hrs. (killed)	7.5 cc.	0	0	marked	0	slight	0	not examined	
672	6*	24 hrs. (killed)	8.5 cc.	0	0	slight	0	slight	0	slight	slight
665	7	24 hrs. (killed)	10.5 cc.	0	slight	slight	slight	0	0	moderate	moderate
673	8	24 hrs. (killed)	0	0	0	slight	slight	0	0	0	slight
664	8†	48 hrs. (died)	8.5 cc.	0	slight	frank tuberculosis		slight	0	moderate	moderate
678	8	48 hrs. (died)	7 cc.	0	0	moderate	slight	0	0	moderate	marked
668	9	24 hrs. (killed)	0	0	0	slight	0	0	0	slight	marked
680	9	4 days (died)	purulent peritonitis	0	0	—	—	—	—	marked	moderate
674	9	1 mo. (killed)	0	0	0	slight	0	0	0	slight	moderate
676	9	1 mo. (killed)	1 cc.	0	0	0	0	0	0	slight	moderate
		B: Controls. Nontuberculous animals given repeated intraperitoneal injections of 5 cc. of from 5–8 per cent O.T.									
689	3	24 hrs. (killed)	0	0	0	0	0	0	0	0	0
687	4	24 hrs. (killed)	0	0	0	0	0	0	0	slight	slight?
685	5	24 hrs. (killed)	0	0	0	0	0	0	0	0	0
682	6	24 hrs. (killed)	0	0	0	slight	0	0	0	slight	0
683	7	24 hrs. (killed)	0	0	0	slight	0	0	0	0	0
688	8	24 hrs. (killed)	1 cc.	0	0	slight	0	0	0	0	0
684	9	24 hrs. (killed)	0	0	0	slight	0	0	0	0	0
690	10	24 hrs. (killed)	0	0	0	slight	0	0	0	0	0

* This animal received one injection of 20 per cent O.T.

† This was the only animal to develop a frank tuberculous peritonitis. The process did not involve pleura or pericardium.

N.B.: In these tables the word "Productive" is used to indicate a diffuse fibrosis and not tubercle-formation.

Fig. 1

Fig. 2

The remaining 7 members of this group received 8 or 9 intraperitoneal injections of tuberculin, and were killed or died 24 or 48 hours after the last injection, or, as in 2 cases, were killed after an interval of one month during which no treatment had been given. Two of the animals that had recently received tuberculin showed abundant peritoneal effusion, two others showed no effusion, while a fifth showed a purulent peritonitis. In two animals, to which no recent tuberculin-treatment had been given, the peritoneal cavities were found to contain only a small or unmeasurable amount of fluid. All twelve of the members of this series were proved by microscopic study to be tuberculous, but the lesions were strictly localized to the inguinal nodes or tissues at the sites of inoculation. In several of the animals that had survived the longest, however, the lesions were inactive. The pleural and pericardial cavities of all these animals were found to show no appreciable increase in fluid. The only gross evidence of thickening of the serous membranes was

Fig. 1. Photomicrograph of visceral pericardium of tuberculous guinea pig (no. 665), showing cellular infiltration and productive changes. Animal killed 24 hours after the seventh intraperitoneal injection of Old Tuberculin.

Fig. 2. Photomicrograph of visceral pericardium of tuberculous guinea pig (no. 678), showing cellular infiltration including a rather dense lymphocytic focus, and productive changes of a more advanced stage than in figure 1. This animal died 48 hours after the eighth intraperitoneal injection of Old Tuberculin.

seen in the pericardium, which in eight of the cases was found to be more opaque than usual (table 2).

Microscopic study of sections from the various serous membranes showed that the pericardial tissues usually participated in the acute inflammatory reactions even when no measurable exudate had been found in the cavities. In about half the animals this was true also of the pleural membranes. Curiously enough, the peritoneum showed relatively little chronic inflammatory change, there being only a slight degree of thickening in the capsules of a certain number of the spleens and livers. Likewise the peritoneal lining of the diaphragm was rarely slightly thickened. The pleurae showed no chronic change at all. Much to our surprise the pericardium showed the most extensive alteration. There was a progressive thickening of parts of both parietal and visceral layers, but particularly the latter, in which the changes were most marked about the auricular appendages. The deeper portions of the thickened visceral pericardium always showed collections of acute and subacute inflammatory cells often infiltrating the substance

Fig. 3

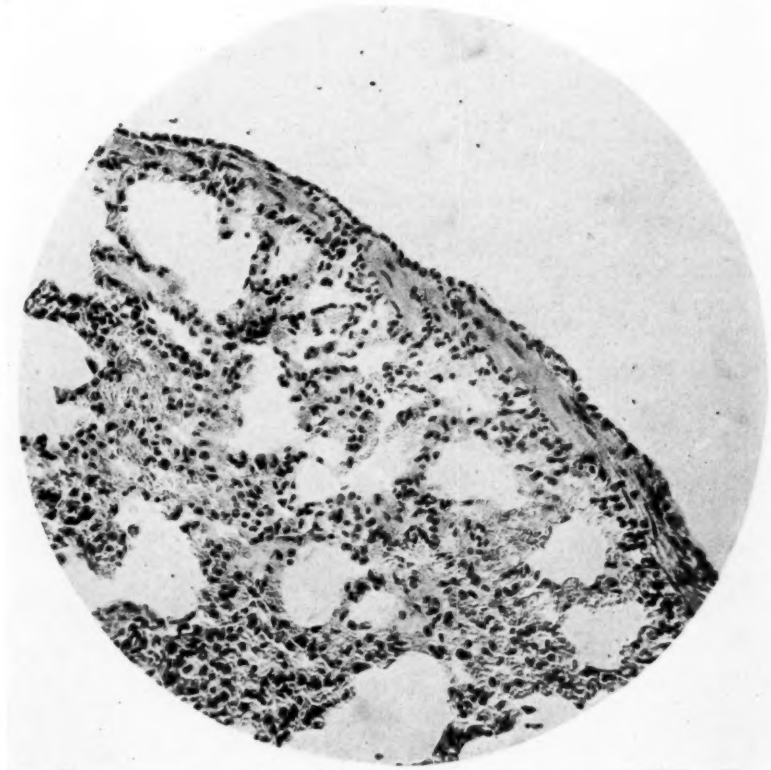

Fig. 4

of the myocardium. These were most often rather diffusely distributed, but in certain locations there were sometimes found rather dense lymphocytic collections. The outer portions were much less cellular. Often fibroblastic and capillary proliferation in this membrane gave the appearance of a thick granulation-tissue layer beneath the serous surface. In cases treated over a longer period of time the picture was that of a chronic fibroid pericarditis. In animals that had received no recent injection of tuberculin the cellular infiltration was much less in evidence. The process bore no resemblance to an organizing surface exudate (figures 1 and 2).

A series of eight nontuberculous guinea pigs was given from 3 to 10 biweekly intraperitoneal doses of 5–8 per cent Old Tuberculin, and none developed any pericardial or other fibrosis (figure 3). Those animals receiving 6 or more such injections did show slight acute reactions in the abdominal cavity which were interpreted as being due to the development of a slight degree of sensitivity (table 2).

Fig. 3. Photomicrograph of parietal and visceral layers of the pericardium of a nontuberculous guinea pig (no. 688), killed 24 hours after the eighth intraperitoneal injection of Old Tuberculin. This is typical of the essentially negative reactions in this series of controls.

Fig. 4. Photomicrograph of the lung of tuberculous guinea pig (no. 752), showing marked fibroid thickening of the pleura. Animal killed 24 hours after tenth intrapleural injection of Old Tuberculin.

Another series of 10 guinea pigs locally infected with the R1 strain of tubercle bacillus was given intrapleural injections of from 3–5 per cent Old Tuberculin every two weeks. Smaller doses were used (1 cc.) since it was found that the animals did not tolerate well the introduction of a larger volume of fluid. The two pleural cavities were used alternately. Dissection of these guinea pigs after varying intervals, in two instances after 10 doses of tuberculin had been administered, showed that effusion was always evoked in both pleural cavities and usually in the peritoneal cavity as well by each injection. Chronic changes were most marked in the visceral pleurae which showed a gradually increasing fibroid thickening on the basis of subacute and chronic inflammation (figure 4). There was but little change of this nature in the peritoneum and even less in the pericardium (table 3).

DISCUSSION

Thus, we have confirmed the findings of others that serous effusions may be elicited in several serous cavities of tuberculous guinea pigs by

TABLE 3

Guinea pigs infected with the R 1 strain of tubercle bacillus and given repeated intrapleural injections of 1 cc. from 3 to 5 per cent O.T.

INFECTED ANIMALS	NUMBER OF INTRA-PLEURAL INJECTIONS	INTERVAL BETWEEN LAST INJECTION AND AUTOPSY	EFFUSION				MICROSCOPIC CHANGES IN SEROUS MEMBRANES					
			Peritoneal	Left pleural	Right pleural	Peri-cardial	Peritoneum		Pleura		Pericardium	
							Exudative	Productive	Exudative	Productive	Exudative	Pro-ductive
725	1	24 hrs. (killed)	1 cc.	0.8 cc.	1 cc.	0	—	—	slight	0	slight	0
717	2	3 days (died)	+	+	+	0	slight	0	0	0	0	0
737	2	24 hrs. (died)	8 cc.	0.3 cc.	0.3 cc.	0	slight	0	slight	0	slight	0
740	2	24 hrs. (died)	3 cc.	0.6 cc.	0.8 cc.	0	slight	0	slight	0	0	0
746	2	24 hrs. (died)	4 cc.	1 cc.	1.5 cc.	0	slight	0	slight	0	0	0
792	2	3 days (died)	+	+	+	0	0	0	0	0	slight	0
794	2	24 hrs. (died)	3.8 cc.	0.4 cc.	0.3 cc.	0	—	—	slight	0	slight	0
735	2	24 hrs. (died)	8.5 cc.	0.5 cc.	0.5 cc.	0	slight	0	0	0	0	0
750	6	24 hrs. (killed)	0.7 cc.	1.8 cc.	0.5 cc.	0	slight	0	slight	slight	0	0
752	10	24 hrs. (killed)	0.5 cc.	0	0	0	slight	slight	slight	marked	slight	0
760	10	15 days (killed)	0	0	0	0	0	slight	0	marked	0	0

the introduction of tuberculin into one of the cavities. The regularity with which allergic sensitivity recurred after eliciting gross allergic reactions in these chronically infected animals contrasts strikingly with the difficulty of maintaining such a state in experimental animals sensitized artificially by the injection of various antigens. It has been shown that well-developed fibrous pericarditis can be produced in mildly infected tuberculous guinea pigs by the repeated injection of tuberculin solutions into the peritoneal cavity. Milder degrees of fibroid change in the peritoneal cavity can be developed under the same circumstances. Chronic fibroid pleuritis can be developed in mildly infected tuberculous guinea pigs by the repeated injection of tuberculin into the pleural cavity. These are obviously examples of scar-formation resulting from allergic reactions the possibility of which has been questioned.

Krause and Willis (9) have noted the development of fibroid change in the pleura and peritoneum following the repeated subcutaneous and intracutaneous injection of living tubercle bacilli (R1 and H37) in guinea pigs that had previously been rendered allergic and more or less immune by inoculation with a strain of organisms of low virulence (R1). The mechanism of this phenomenon has never been explained. Some of these animals exhibited no visceral tuberculosis. In the course of their repeated skin inoculations the animals developed a whole series of local skin reactions which, by the way, exhibited a period of much lessened activity during a certain phase of the experiment. These animals, therefore, were subjected to repeated allergic reactions. Whether the systemic effect of these reactions resulted in the fibrosis noted is an interesting question for speculation. Our approach to the problem of the relation of repeated allergic reactions as a cause of fibroid serositis was direct, but may nevertheless depend upon the same factors.

We found that the more heavily infected the tuberculous guinea pigs the more extensive and severe was the allergic serositis produced (7) (8). The reaction, when severe, often proved fatal in some 12 to 24 hours and under such circumstances the tuberculous lesions were found to be the seat of great congestion and acute cellular infiltration (focal reaction). The sites of recent and subsiding skin tests became inflamed again and sometimes necrotic as would be expected. Of course no deaths from acute anaphylaxis were observed (Baldwin (10)).

To return to a consideration of clinical polyserositis in man, it is true that in this condition adhesions between the serous surfaces are often found. In fact, death is frequently caused by the hampering

effect upon the heart's movements of these adhesions or by cicatricial tamponade of the heart. In the guinea pigs in our experiments but few adhesions developed. They did develop, or start to develop, however, in the animals into which Paterson injected living cultures of tubercle bacilli, representing, in these cases, the organization of surface exudates and the development of tubercles. The degree of inflammatory reaction and the presence or absence of actual infection in the serous cavities apparently condition this phenomenon.

It is planned to repeat these experiments carrying them on over longer periods, in an endeavor to see to what extent a fibroid polyserositis can be developed in this manner.

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STUDIES ON THE DISSOCIATION OF TUBERCLE BACILLI WITH SPECIAL REFERENCE TO THE AVIAN AND HUMAN TYPES[1,2]

ELEANOR ALEXANDER-JACKSON

The S colonies of dissociated bacteria are usually described as round, smooth-edged, moist, convex, and glistening, and readily shaken or rubbed into a homogeneous suspension in physiological saline solution. Petroff was the first to obtain S colonies of avian and bovine tubercle bacilli. The *BCG* and human tubercle-bacillus colonies, which he calls "S," however, do not correspond to the usual S type. According to descriptions and photographic plates, they appear as flat, dull, wrinkled, and irregular in outline. These so-called "S" colonies have been obtained since by others, and although they are more easily emulsified in saline solutions and are of somewhat greater virulence than R colonies, they seem to be intermediates rather than true S.

Behner reports occasional smooth, moist, hemispherical *BCG* colonies on Petroff's medium, which were unstable in subsequent generations. A few writers have described moist, round, smooth colonies obtained from tuberculous sputum. These tubercle bacilli may have been of the human type. However, it is generally acknowledged that the human tubercle bacillus is difficult to dissociate completely, and there has been no definite description of true human type-S colonies.[3]

An attempt was made to dissociate several human strains to true S colonies, and, in addition, to develop or modify a medium which would favor the continued formation of S colonies so that they might be grown in pure culture. S colonies of the smooth, round, convex, glistening type were obtained (*cf.* E. Alexander, 1934), and appear to maintain themselves in pure culture on a modified medium after more than eight gen-

[1] From a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at New York University, June, 1934.

[2] The completion of this paper was made possible by a grant from the Rosenwald Family Association.

[3] While this paper was being prepared for publication, a paper appeared by K. Smithburn (*Jour. Exp. Med.*), describing human type S colonies obtained from sputum and other sources, grown on Cooper's egg-yolk medium.

erations. Avian tubercle bacilli, because of the ease with which they are dissociated, were employed as a guide in preliminary experiments.

DISSOCIATION OF HUMAN TUBERCLE BACILLI ON MODIFIED BORDET-GENGOU MEDIUM

Williams observed that the Bordet-Gengou medium, employed for the isolation of *B. pertussis*, was also a good nutrient for tubercle bacilli. The excellence of this medium for the growth and isolation of tubercle bacilli from sputum or tissue has been clearly demonstrated by Mishulow (1932). She found that tubercle bacilli of avian, bovine, human, and *BCG* strains produced characteristic colonies on this medium. The Bordet-Gengou medium was tried out for the study of R and S colonies after other media had proved unsatisfactory for the human S. It has proved most valuable for dissociation work, as it not only can be modified for growing R or S colonies, but is also suitable in unmodified form as a control medium on which the undissociated culture maintains characteristic colonies. It is also valuable in observing dissociation in the animal body, as primary isolation colonies from the organs of animals inoculated with R or S, will maintain, for at least one generation, the colony form induced by animal passage. Mishulow had also shown that primary-isolation colonies were not always "typical" Bordet-Gengou colonies, although on subsequent plantings they usually became so.

In terms of a scale, colonies may range roughly as follows: R—Rs—RS—SR—Sr—S, the symbol being indicative of the degree of roughness or smoothness of the colony. Which colony type will predominate appears to depend on the kind of medium used and the physical state of both medium and inoculum (*cf.* tables 1 and 2). Sufficient moisture is of the utmost importance in obtaining and maintaining S colonies. The previous environment of the inoculum naturally has an influence on the organisms. A culture obtained from one organ of an animal may give a different type of colony from that obtained from another organ, although the animal be inoculated with one type only. This will be illustrated later. In general, unfavorable growth conditions discourage the S, while the R may still flourish.

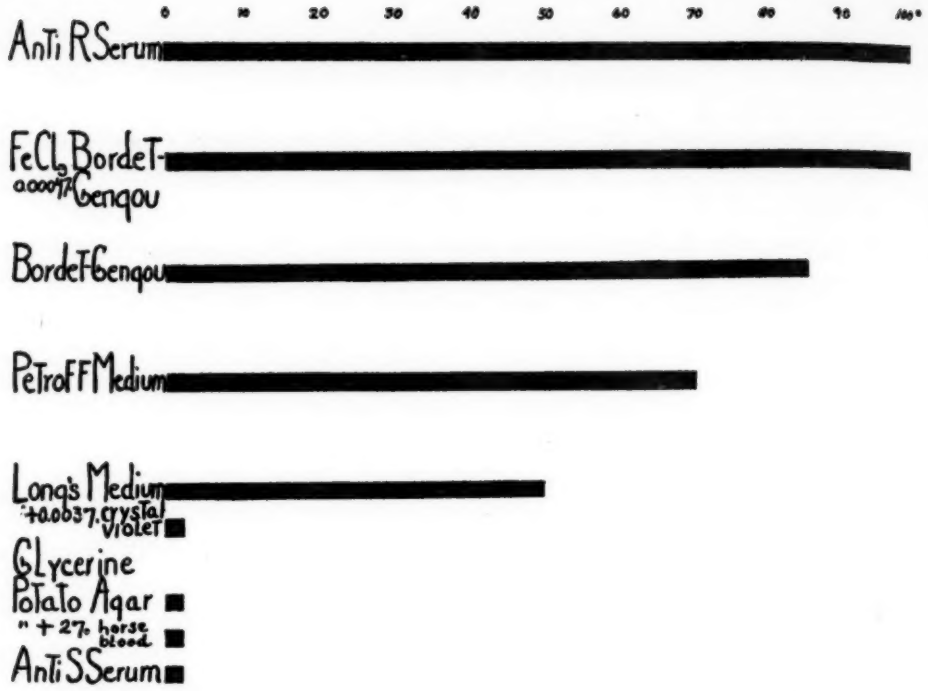
"Typical" human tubercle-bacillus colonies on Bordet-Gengou medium tend to be convex, with irregularly indented bases, and are finely granular in texture, some appearing dull, and others shiny. These colonies in terms of the above scale seem nearer the S than the R, pos-

sibly varying between SR and Sr. Avian S colonies, in my experience, have been stable on Bordet-Gengou medium. Not even an intermediate has been found in the strain used. The R cultures, on the other hand, often gave rise to intermediate or S colonies. My experience with bovine strains has been too limited to discuss the colonies from this angle. The bovine colonies obtained by Mishulow on Bordet-Gengou medium appear to be near to the S type. My observations suggest, furthermore, that the nearer the colonies approach to the extreme ends of the scale, the more the different types resemble one another. The true S of human origin very closely resembles the avian S. Graphs 1 and 2 attempt to give a rough estimate of the relative ability of various media to support S colonies of human and avian tubercle bacilli.

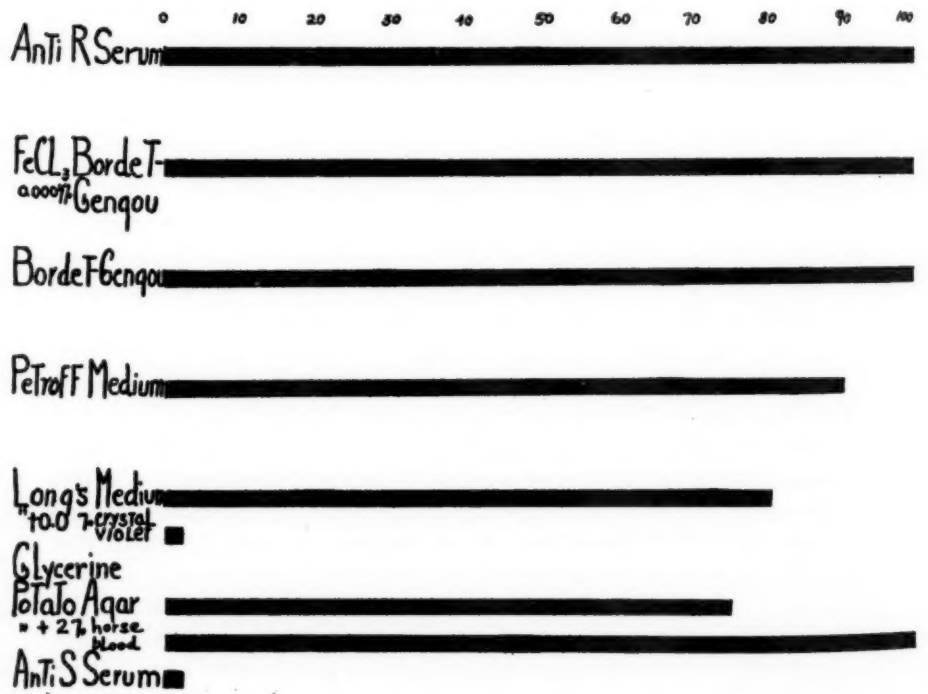
The instability of human-type S colonies on other than anti-R sera or certain normal sera suggested that even so enriched a medium, as is the Bordet-Gengou, might be lacking in, or contain insufficient amounts of certain specific substances needed to favor the stability of human S colonies. Since anti-R sera are not readily obtained in large amounts, it seemed more reasonable to determine whether or not some more readily obtained substance could be added to Bordet-Gengou medium which would produce a "typical" colony of the S type. To obtain individual colonies, Petroff's dilution and filtration technique was employed.

A loopful of culture was placed in a sterile 25-cc. glass bottle containing 3 cc. of sterile distilled water and several glass beads. The bottles were tightly stoppered with sterile solid rubber stoppers, and were shaken by hand until fairly homogeneous suspensions were obtained. The suspensions were filtered through two layers of Whatman no. 5 filter paper to remove clumps. The filters were made from pyrex test tubes, 3/4-inch diameter, and each contained two small paper funnels. The filters were fitted into test tubes of the same size, and held in position by adhesive tape. The filters were plugged with cotton, and the combined filters and tubes were wrapped in paper and sterilized at 165°C. in a hot-air sterilizer.

The filtered suspensions were diluted with 5 cc. of distilled water. Smears were made by allowing one drop of suspension from a one-cc. pipette to dry on a clean glass slide. If the smear showed more than one or two bacilli every few fields, under a 1/12 oil-immersion lens, the suspensions were diluted further. One or two drops of suspension were then delivered to each slant of medium. Slants were used rather than plates, as they were less liable to contamination under crowded working conditions, and satisfactory colonies could be obtained. The slants were sealed with cotton plugs dipped in hot paraffin, and all slants

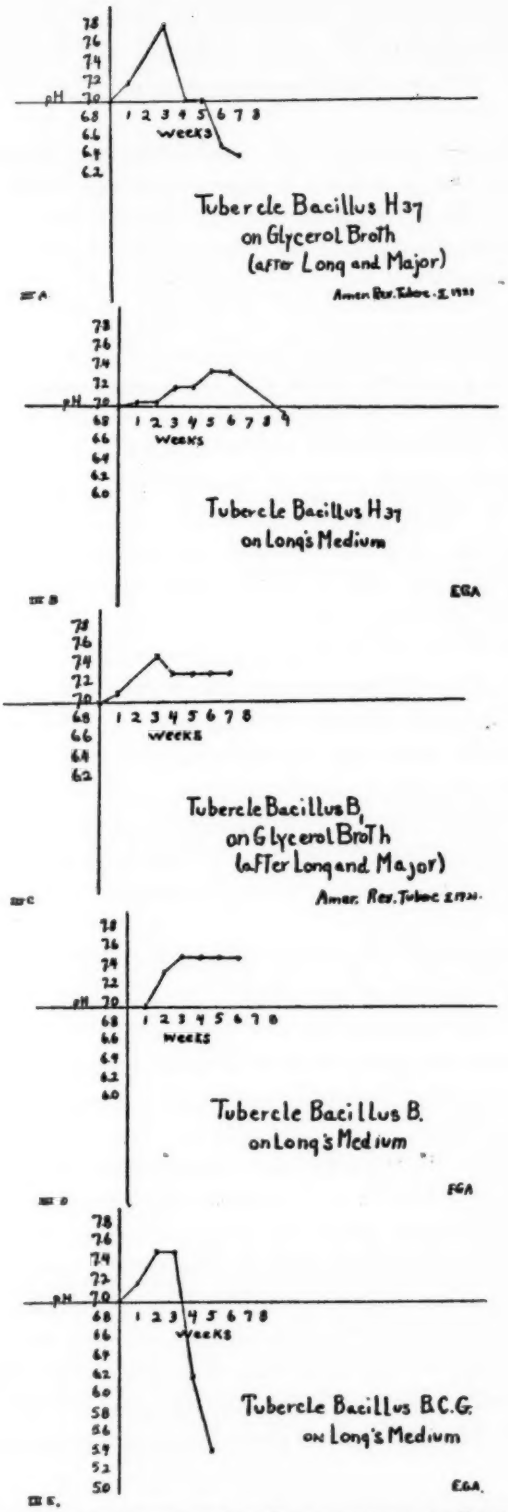


GRAPH 1. THE RELATIVE ABILITY OF VARIOUS MEDIA TO SUPPORT S COLONIES OF HUMAN TUBERCLE BACILLI



GRAPH 2. THE RELATIVE ABILITY OF VARIOUS MEDIA TO SUPPORT S COLONIES OF AVIAN TUBERCLE BACILLI

* Figures 0–100 indicate degree of S characteristics.



GRAPH 3. CHANGES IN HYDROGEN-ION CONCENTRATION OF LONG'S SYNTHETIC MEDIUM AS COMPARED WITH CHANGES ON GLYCEROL-BROTH AS SHOWN BY LONG AND MAJOR, 1921

were incubated for 6 weeks at 37°C. The tubes were examined under a low power microscope for growth and colony morphology, and a reading for each tube recorded. The proportion of blood to glycerine-potato-agar base was one part of blood to three parts of base. The final pH was about 6.8.

1. Experiments with Avian Strains

A dissociated avian strain was selected for a guide strain since, as already mentioned, avian bacilli are much more easily dissociated than are human strains. A preliminary planting of both R and S was made on unmodified Bordet-Gengou slants to note any variations or dissociations. The S colonies showed no variations. In 4 tubes out of 12 seeded with R organisms, however, the colonies were all distinctly rounder, smoother, and moister than the colonies in the other tubes. In 3 other tubes there were a few of these intermediate colonies. The medium in all the slants came from the same bottle, and the same amounts of medium and inoculum were measured out into each tube with pipettes. Growth was equally luxuriant in all the tubes. These tubes were new, glass tubes, and had been washed, neutralized with dilute hydrochloric acid, and thoroughly rinsed. One possible variable considered was imperfect distribution of red blood corpuscles. Care was taken to mix the blood well; nevertheless, it seemed possible that even slight unevenness of distribution of some substance in the medium might be sufficient to influence colony morphology. The tubes were all well sealed.

A second possibility considered was the diffusion of ions from the glass of the tube in amounts sufficient to affect the organisms. It is well known that infinitesimal traces of simple chemicals can exert marked effects on inorganic, organic and life processes. Diffusible substances often present as impurities in glass are salts of iron and manganese.

A trial experiment was carried out to determine whether or not small amounts of ferric chloride or manganese sulphate, added to Bordet-Gengou medium, would cause any change in colony morphology. One gram each of $FeCl_3(6H_2O)$ and crystalline $MnSO_4$ $(4H_2O)$ were weighed out on a quantitative balance and added to 100-cc. amounts of sterile distilled water (pH 6.0). These solutions were each diluted 1 to 9 three times with distilled water, which had been measured with aseptic precaution into sterile tubes. Of the last dilutions 6.5 cc. amounts were measured out to 50 cc. of melted glycerine-potato-agar base, mixed, and

then 15 cc. of sterile citrated horse-blood was added, making the final concentration of manganese salt about 0.0001 per cent and that of the hygroscopic iron salt about 0.00004 per cent. $FeCl_3$, due to its hygroscopic nature, had to be titrated with standard potassium iodide and sodium thiosulphate in order to determine the exact amount of $FeCl_3$. A concentrated solution of $FeCl_3$ supposedly 15 per cent actually contained but 6.1 per cent.[4] In future experiments a stock solution of titrated concentrated $FeCl_3$ was kept in the ice-box, and dilutions made from this solution.

Method of Titrating Stock Ferric-Chloride Solution (United States Pharmacopeia, X, 1926)

1: 15 gm. of ferric chloride (C. P.) were weighed out into a clean glass bottle with a rubber stopper, and 100 cc. distilled water were added. The solution was thoroughly mixed and allowed to stand overnight in the ice-box.

2: Using a volumetric pipette, 10 cc. of the above solution were placed in a 250-cc. erlenmeyer flask; 25 cc. distilled water; 3 cc. concentrated HCl; and 4 gm. potassium iodide added (10 cc. of a 40 per cent solution).

3: The erlenmeyer was closed with a clean rubber stopper, and the fluid was agitated, and allowed to stand for 30 minutes at 37°C. The flask was then cooled down to room temperature, and 100 cc. distilled water added. The free iodine was then titrated with 0.1 N standard sodium thiosulphate. Of a 1 per cent starch-solution, 2 cc. was used as an indicator. The thiosulphate was added slowly until the yellow color of the iodine was almost gone. Then the starch was added, and more thiosulphate, until the deep blue color was completely bleached.

4: 1 cc. of 0.1 N $Na_2S_2O_3$ is equivalent to 0.01622 gm. $FeCl_3$, and 0.005584 gm. of Fe. This figure was corrected by multiplying by the standardization factor of the $Na_2S_2O_3$ used (0.867). The corrected figures for ferric chloride were 0.0141 gm., and for iron 0.0048 gm.

5: The average burette readings of $Na_2S_2O_3$ added to the Iodine was 43.08 cc. This was multiplied by 0.0048 giving 0.21 gm. of Fe in 10 cc. of $FeCl_3$ or 0.021 gm. of iron per cc. The same figure multiplied by 0.0141 gave 0.61 gm. of $FeCl_3$ in 10 cc. 100 cc. of this solution would then contain *6.1 per cent* $FeCl_3$, rather than 15 per cent.

[4] Baker's C. P. $FeCl_3$ was used. A freshly opened bottle gave an 8 per cent solution.

6: Experience showed that Bordet-Gengou medium containing 0.0004 per cent of $FeCl_3$ gave the best results with human tubercle bacilli. Therefore dilutions were calculated as follows:

1 cc. : 6 per cent as x cc. : 4 per cent

0.67 cc. were diluted with 9.3 cc. of sterile distilled water placed in a sterile tube. This gave a 0.4 per cent solution. Of this solution, 1 cc. added to 9 cc. water in a second tube gave a 0.04 per cent solution. This was again diluted 1 to 10, giving a dilution of 0.004 per cent. To 65 cc. of medium were added 6.5 cc. of 0.004 per cent solution, giving approximately a final dilution of 0.0004 per cent in the medium as desired. This dilution technique although crude, has been found practical from a bacteriological standpoint, since the S colonies are obtained, and with a minimum of contamination.

The results of the trial experiment with iron and manganese salts are expressed in table 1.

TABLE 1

STRAIN	MEDIUM	RESULTING COLONIES
Avian R	Bordet-Gengou	None of the tubes had S
Avian R	Bordet-Gengou plus 0.00004 per cent $FeCl_3$	4 of 6 tubes almost true S
Avian R	Bordet-Gengou plus 0.0001 per cent $MnSO_4$	3 of 6 tubes had SR
Avian S	Bordet-Gengou	All S
Avian S	Bordet-Gengou plus 0.00004 per cent $FeCl_3$	All S
Avian S	Bordet-Gengou plus 0.0001 per cent $MnSO_4$	All S

Dissociation from R to S appeared to have been stimulated by both metal salts, the better results being given by $FeCl_3$.

An extensive experiment was then carried out with other metal salts, variations in pH, medium, and variations in concentration of iron and manganese. Crystal-violet was added to Bordet-Gengou medium in order to compare its effect in this medium with its effect in Long's medium, in which certain small amounts were found to inhibit S organisms. The writer believes that the 1 part in 30,000 of gentian-violet in Petroff's medium while supporting avian S colonies, will not allow the formation of human S colonies. The results of this experiment are given below in table 2.

TABLE 2

MEDIUM	pH	AVIAN R		AVIAN S	
		Number of slants	Colonies	Number of slants	Colonies
Bordet-Gengou	6.8	6	R	3	S
	7.0	6	R	3	S
	7.4	6	R	2	S
Bordet-Gengou plus $FeCl_3$ 0.00004 per cent	6.8	5	RS	6	S
		1	R		
	7.0	6	R	6	S
	7.4	3	Rs	6	S
		3	R		
Bordet-Gengou plus $FeCl_3$ 0.0004 per cent	6.8	2	S	6	S
		2	Rs		
		2	R		
	7.0	1	S	6	contaminated
		2	Rs		
		2	R		
	7.4	4	Rs	6	S
		2	R		
Bordet-Gengou plus $FeCl_3$ 0.004 per cent	6.8	4	S	6	S
		1	Rs		
		1	contaminated		
	7.0	6	R	6	S
	7.4	6	R	6	S
Long's Medium	6.8	6	R	6	S
Long's Medium plus $FeCl_3$ 0.0004 per cent	6.8	6	Rs	6	S
Bordet-Gengou plus $CuSO_4$ 0.0001 per cent	6.8	1	S	6	S
		3	Sr, RS		
		1	contaminated		
Bordet-Gengou plus $CuSO_4$ 0.001 per cent	6.8	4	RS	3	S
		1	Rs	3	contaminated
		1	R (mold)		
Bordet-Gengou plus $CuSO_4$ 0.00001 per cent, $FeCl_3$ 0.00004 per cent	6.8	5	R	5	S
Bordet-Gengou plus $MnSO_4$ 0.0001 per cent	6.8	1	Sr	6	S
		1	RS, R		
		2	R		

TABLE 2—*Concluded*

MEDIUM	pH	AVIAN R		AVIAN S	
		Number of slants	Colonies	Number of slants	Colonies
Bordet-Gengou plus $MnSO_4$ 0.001 per cent	6.8	1 4	S RS	6	S
Bordet-Gengou plus $ZnSO_4$ 0.0001 per cent	6.8	6	contaminated	6	contaminated
Bordet-Gengou plus $ZnSO_4$ 0.001 per cent	6.8	4 2	Rs (small) R	6	S
Bordet-Gengou plus $NiCl_3$ 0.0001 per cent	6.8	5 1	Rs, R R	4	S
Bordet-Gengou plus crystal-violet 0.001 per cent	6.8	6	R	6	S
Bordet-Gengou plus crystal-violet 0.01 per cent	6.8	1 5	S RS	6	S

The conclusions drawn from these experiments are as follows:

1. Although S and intermediate colonies were obtained with the addition of other metallic salts, ferric chloride gave the best results. In this experiment, the metals in order of their ability to cause R to S dissociation appeared to be iron, copper, manganese, zinc, and nickel.
2. Ferric chloride was not effective in an alkaline medium.
3. Ferric chloride gave better results with Bordet-Gengou than with Long's medium.
4. The best results with ferric chloride were obtained with a concentration of 0.004 per cent. This concentration contains 14 mgm. of iron per litre, or 40 mgm. $FeCl_3$ per litre.
5. Crystal-violet, added to Bordet-Gengou in the above amounts, did not cause any dissociation from S to R. On the contrary, a concentration of 0.01 per cent appeared to induce some S like colonies on tubes seeded with R.

In a paper on the *Bacteriostatic Action of Gentian Violet and Its Dependence on the Oxidation-Reduction Potential*, Ingraham (1933) stated that the bacteriostatic action of gentian-violet is a function of its ability to poise the oxidation-reduction potential of the medium, and that this dye benefits growth in media in which its presence induces a more favorable potential. Ingraham worked with *B. coli* in broth. Nevertheless, this conception may bear some relation to the difference in behavior of crystal-violet added to Long's medium, and the same dye added to Bordet-Gengou medium. It is possible that the dye is reduced by the blood present in the latter. It is, however, difficult to obtain

an accurate idea of physicochemical conditions at the surface of a solid medium when the presence of atmospheric oxygen enters into the picture.

The results obtained in two additional experiments with the avian strain are given in table 3. Observations were made for possible colony variations from S to R. The effect of cystein and glutathione was tried out, as these compounds were found to stimulate growth and development of marine embryos (Hammett and Hammett (1932)). (*Cf.* table 3.)

TABLE 3

MEDIUM	pH	AVIAN R		AVIAN S	
		Number of slants	Colonies	Number of slants	Colonies
Long's agar plus 10 per cent horse blood	6.8	4	R	6	S
	7.1	5	R	—	scant growth
	7.4	5	R	6	S
		1	contaminated		
Bordet-Gengou	6.8	7	R	7	S
Bordet-Gengou plus cystein (5×10^{-7} M/L of sulphur)		4	R, Rs	10	S
		2	moldy		
Bordet-Gengou plus glutathione (5×10^{-7} M/L of sulphur)		5	R, Rs		
		1	R	7	S

No variation of S was observed, and very little of R.

2. *Experiments With Human Tubercle Bacilli*

The next experiments were made with two recently isolated strains H3 (Mishulow), and Arilotta (Alexander). Successive generations on modified and unmodified Bordet-Gengou medium were observed, in order to separate out three distinct varieties from each, namely an undissociated, an S, and an R. Since ferric chloride had given the best results in R to S dissociation of avian bacilli, various concentrations of this substance were tried out in the hope that similar results would be obtained with the human type. The results are expressed in tables 4 to 7. (*Cf.* plate 1, figures 1A, B & C and 2A, B & C.)

The most S-like colonies were fished from slants containing ferric chloride, and again planted on Bordet-Gengou slants containing ferric chloride. R-like growth was planted on slants containing crystal-violet, and the control bacilli were kept on plain Bordet-Gengou medium.

TABLE 4

Successive generations of human tubercle bacilli grown on modified and unmodified Bordet-Gengou medium

Generation 1

MEDIUM	H3		ARILOTTA	
	Number of slants	Colonies	Number of slants	Colonies
Bordet-Gengou (pH	7	typical Sr or SR human (Mishulow)	6	typical human SR
	3	flat, waxy curled edges	3	round, smooth edges, dull
			2	flat, irregular opaque
Bordet-Gengou plus $FeCl_3$, 0.00004 per cent	4	flat, waxy curled edges	4	*S moist, convex
			1	Sr
Bordet-Gengou plus $FeCl_3$, 0.0004 per cent	1	more convex, smoother	9	*S
	6	flat, waxy, curled edges	1	Sr
Bordet-Gengou plus crystal-violet, 0.0001 per cent	6	flat, waxy curled edges	9	typical human SR
	2	R	1	flat, irregular edges

* Indicates the first appearance of true S colonies.

TABLE 5

Generation 2

MEDIUM	H3		ARILOTTA	
	Number of slants	Colonies	Number of slants	Colonies
Bordet-Gengou	3	typical human SR	2	typical human
	3	Sr	1	Sr
Bordet-Gengou plus $FeCl_3$, 0.0004 per cent	9	Sr	7	S
	1	SR	1	flat, dull (medium dry)
Bordet-Gengou plus $FeCl_3$, 0.004 per cent	—	—	2	S
			6	SR tops tend to be flat
Bordet-Gengou plus crystal-violet, 0.0001 per cent	3	SR	5	intermediates Rs
	2	SR; a few RS		
Bordet-Gengou plus crystal-violet, 0.001 per cent	4	SR	5	RS
	2	contaminated		
Bordet-Gengou plus crystal-violet, 0.006 per cent	3	RS	3	RS
	1	SR	3	no growth

Ferric chloride in a concentration of 0.0004 per cent appeared to be promising for use with the human-type tubercle bacilli. This concentration contains 1.4 mgm. of iron per litre and 4 mgm. of $FeCl_3$ per litre. The most S-like colonies were again fished and planted on

TABLE 6

Generation 3

MEDIUM	H3		ARILOTTA	
	Number of slants	Colonies	Number of slants	Colonies
Bordet-Gengou	7	typical human SR	3	RS flat, irregular, raised centre
	2	small, flat Rs	2	no growth
			1	moldy
Bordet-Gengou plus $FeCl_3$, 0.0004 per cent	2	*S convex, shiny	6	S
	5	small flattish Rs (medium dry)		

* Indicates the first appearance of true S colonies.

TABLE 7

Generation 4

MEDIUM	H3		ARILOTTA	
	Number of slants	Colonies	Number of slants	Colonies
Bordet-Gengou	1	S	6	SR cheesy texture, some with raised split centres
	4	SR		
Bordet-Gengou plus $FeCl_3$, 0.0004 per cent	9	S	8	S
			1	no growth
Glycerine-potato-agar	5	no growth	5	no growth
Glycerine-potato-agar plus 3 per cent horse blood	8	R, Rs	11	R, Rs
	2	no growth	1	no growth

ferric chloride Bordet-Gengou. It was also found that R colonies could be maintained on Bordet-Gengou medium base containing but 3 per cent of whole blood.

The fourth generation on ferric-chloride Bordet-Gengou medium gave

100 per cent S colonies with both the H3 and Arilotta strains. The eighth generation has continued to produce S colonies. It was noted that sufficient moisture plays a very important part in the formation of S colonies. Dryness, or the presence of a contamination in the tube will often cause a roughened type of colony. The ageing of a culture results in a certain amount of loss in water by the organisms. This may be a factor in the dissociation of aged cultures.

PLATE 1

Reading from left to right: figs. 1A, B & C. Top row: figs. 2A, B & C. Second row: figs. 3A, B & C. Third row: figs. 3D, E & F. Bottom row: figs. 3G; 4A & B.

Fig. 1A. Dissociated human tubercle bacilli Rs (Arilotta strain) on glycerine-potato-agar 5 per cent horse-blood
1B. Undissociated human tubercle bacilli (Arilotta strain) on Bordet-Gengou medium
1C. Dissociated human tubercle bacilli S (Arilotta strain) on Bordet-Gengou medium plus 0.0004 per cent ferric chloride

Fig. 2A. Dissociated human tubercle bacilli Rs (H3 strain) on glycerine-potato-agar plus 5 per cent horse-blood
2B. Undissociated human tubercle bacilli (H3 strain) on Bordet-Gengou medium
2C. Dissociated human tubercle bacilli S (H3 strain) on Bordet-Gengou medium plus 0.0004 per cent ferric chloride

Fig. 3A. Avian S on Bordet-Gengou
3B. Bacilli cultured from organs of a mouse inoculated with S avian (spleen tissue), pure S
3C. Intestine: note one large R colony among small S colonies
3D. Kidney: smooth coiled colonies S, Sr
3E. Liver: flattish intermediate SR
3F. Peritioneum: Rs
3G. Spleen (pus): R

Fig. 4A. H37 R pure rough nodule colonies from the inguinal lymph nodes of a guinea pig inoculated with corrugate growth from Long's fluid medium, on Bordet-Gengou medium
4B. H37 S and intermediate colonies from the lymph nodes of a guinea pig inoculated with membrane growth from Long's fluid medium, on Bordet-Gengou medium

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Dissociation of Avian and Human Tubercle Bacilli by Animal Passage

1. Avian: Cultures were made on Bordet-Gengou medium directly from the organs of white mice which were killed 4½ months after being inoculated intraperitoneally with avian S and avian R tubercle bacilli (*cf.* table 8 and plate 1, figures 3A–G).

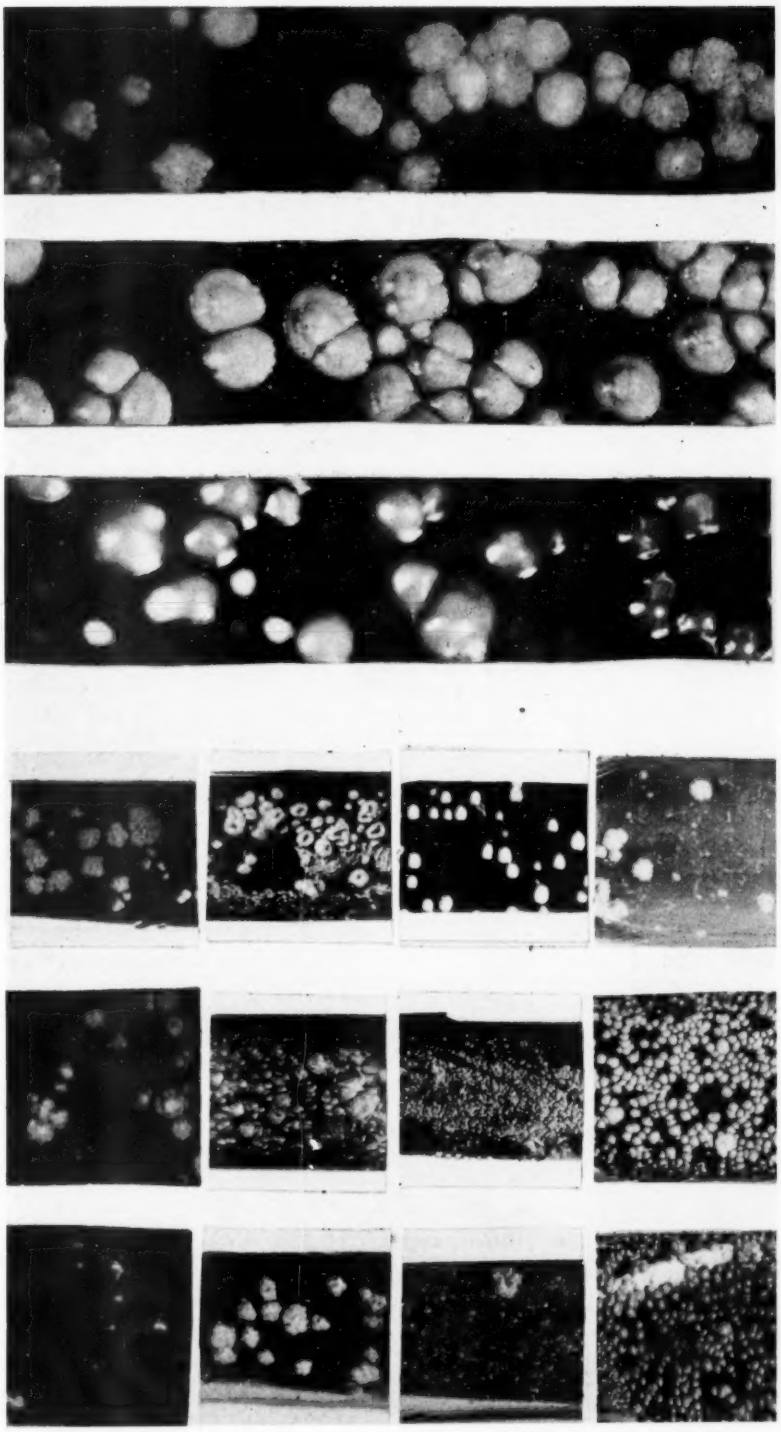

PLATE 1

The general tendency seemed to be for the organisms to go towards the R side of the scale rather than the S. It is interesting to note that both extreme S and extreme R were obtained from a single organ, the spleen in this case. It is probable that conditions in pus were less favorable than the conditions in more recently invaded tissue.

2. Human: The strain H37 was dissociated to true S and intermediate colonies by passage through the inguinal lymph nodes of a guinea-pig. The inoculum injected into the inguinals was thin-membrane growth

TABLE 8

ORGAN	AVIAN S 1.0 MGM.	AVIAN R 1.0 MGM.
	Colonies	Colonies
Peritoneum	Rs or R	Rs
Heart's blood	RS, Rs	R
Spleen	R (pus) S, a few R (tissue)	Rs
Liver	Rs	contaminated
Kidney	Sr, S	R
Lung	contaminated	RS
Intestine	R, S, Sr	contaminated

TABLE 9

Guinea pig 4915 : 1.0 mgm. H3 S

ORGAN	COLONIES
Spleen	S, a few pigmented R
Liver	S, Sr, a few R
Peritoneum	R, RS, Sr
Guinea pig 2427 : 0.01 mgm. H3 S (Cf. plate 2, figures 2A & B)	
Inguinal lymph nodes	S, Sr
Spleen	SR

from Long's fluid medium. The dose was 1.0 mgm. Cultures made from the guinea pigs were plated out on Bordet-Gengou medium.

Direct cultures on Bordet-Gengou medium were also made from guinea pigs inoculated intraperitoneally with H3 S (*cf.* table 9). The tissues were not treated with 3 per cent NaOH in the usual manner of isolating tubercle bacilli from sputum and tissue as it was found that true S were not obtained when the method was employed. Smithburn also states that he found NaOH and 6 per cent H_2SO_4 inhibitory to S formation.

Extreme S and R are both isolated from the liver. There is the same apparent tendency for the S to change toward the R type. As with the avian S inoculated into the mouse, as described above, the colonies obtained from the peritoneum were predominantly R-like. This finding may have some relation to the fact that few acid-fast rods are found in the peritoneum in tuberculous infection, unless the infection be particularly severe. Conditions in that region appear to be unfavorable to the organism.

Dissociation on Long's Medium

As observed by others, including Petroff, Reed and Rice, Kahn, and Behner, tubercle bacilli were found to undergo dissociation when grown on liquid media. On Long's synthetic medium patches of rough corrugated pigmented R growth would often be surrounded by a very thin whitish film. This film, if transplanted to fresh medium, would tend to thicken up as it grew, and would often acquire deeper pigment and a typical R appearance. When the film was transplanted to Long's agar it usually produced flat, irregularly-edged, grayish-white, dull colonies, considered by Petroff, Birkhaug, and Behner to be a type of S, but which the writer prefers to call an intermediate type. The corrugated thick growth invariably produced R on solid medium.

Membranous growth of the human strain H37 was taken from Long's medium and inoculated subcutaneously into guinea pigs. Cultures from the inguinal lymph nodes of one of these guinea pigs made on Bordet-Gengou medium gave a mixture of intermediate colonies and some true round moist S. Corrugated growth from the same culture on Long's medium, likewise inoculated into guinea pigs, gave on Bordet-Gengou medium only knotty R colonies. Dissociation of the *BCG* strain was also observed on Long's medium. Illustrations of growth on Long's medium, and of the dissociated human colonies are shown in plate 2, figures 1A, B & C.

The writer agrees with Kahn that the characteristics of growth-membranes on liquid media are a less accurate criterion for dissociation than individual colony morphology on a solid medium, since film growths produce colonies which may vary in their degree of roughness or smoothness.

Although, on Long's medium, a pH of about 6.6 to 6.8 seemed to favor the R type whereas a pH of 7.0 or 7.2 favored dissociation of R into membrane growth, it was seldom that the entire culture consisted of one

type of growth. An attempt was made with dissociated avian tubercle bacilli to determine whether or not the pH curve produced by growth of the R type differed from that produced by the S type. The R, and still more the S, were too unstable on this medium to permit any results. A number of pH curves were made on Long's medium to determine whether or not bovine, human, and *BCG* gave characteristic curves, and whether or not the first two types corresponded to the pH curves obtained by others on glycerol broth. The graphs indicate that the curves

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PLATE 2

Reading from left to right: figs. 1A, B & C. Top row: figs. 2A & B and 3A. Middle row: figs. 3B, C & D. Bottom row: figs. 3E, F & G

Fig. 1A. Avian tubercle bacilli on Long's fluid medium; rough and membrane growth
1B. *BCG* (Bacillus Calmette-Guérin) on Long's fluid medium; rough, intermediate, and membrane growth
1C. Human tubercle bacilli (H37) on Long's fluid medium; rough, intermediate, and membrane growth

Fig. 2A. Human tubercle bacilli (H3) from the inguinal lymph nodes of a mouse inoculated with 0.01 mgm. of S, showing little dissociation
2B. Human tubercle bacilli (H3) from the spleen of the same mouse, showing more dissociation. Colonies resemble characteristic of colonies of the human type cultivated on Bordet-Gengou medium. The medium in both cases is Bordet-Gengou unmodified

Fig. 3A. Avian tubercle bacilli R on Long's agar
3B. *BCG* R on Long's agar
3C. *BCG* RS (similar to Petroff's *BCG* S) on Long's agar
3D. Bovine (B1) tubercle bacilli on Long's agar showing nodular R and veil-like RS
3E. Human tubercle bacilli (H37) R on Long's agar
3F. H37 intermediate colonies Rs or RS on Long's agar
3G. H37 veil-like growth SR derived from Long's fluid medium, on Long's agar

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on Long's medium are similar to those obtained on nonsynthetic broth. The curves given by the *BCG* were human rather than bovine in character.

Attempts to Dissociate Human Strains with Sera Obtained from Tuberculous and Nontuberculous Persons

The question presented itself of whether or not sera from tuberculous patients would be able to dissociate human tubercle bacilli, and whether or not the power of a serum to dissociate from R to S bore any relation

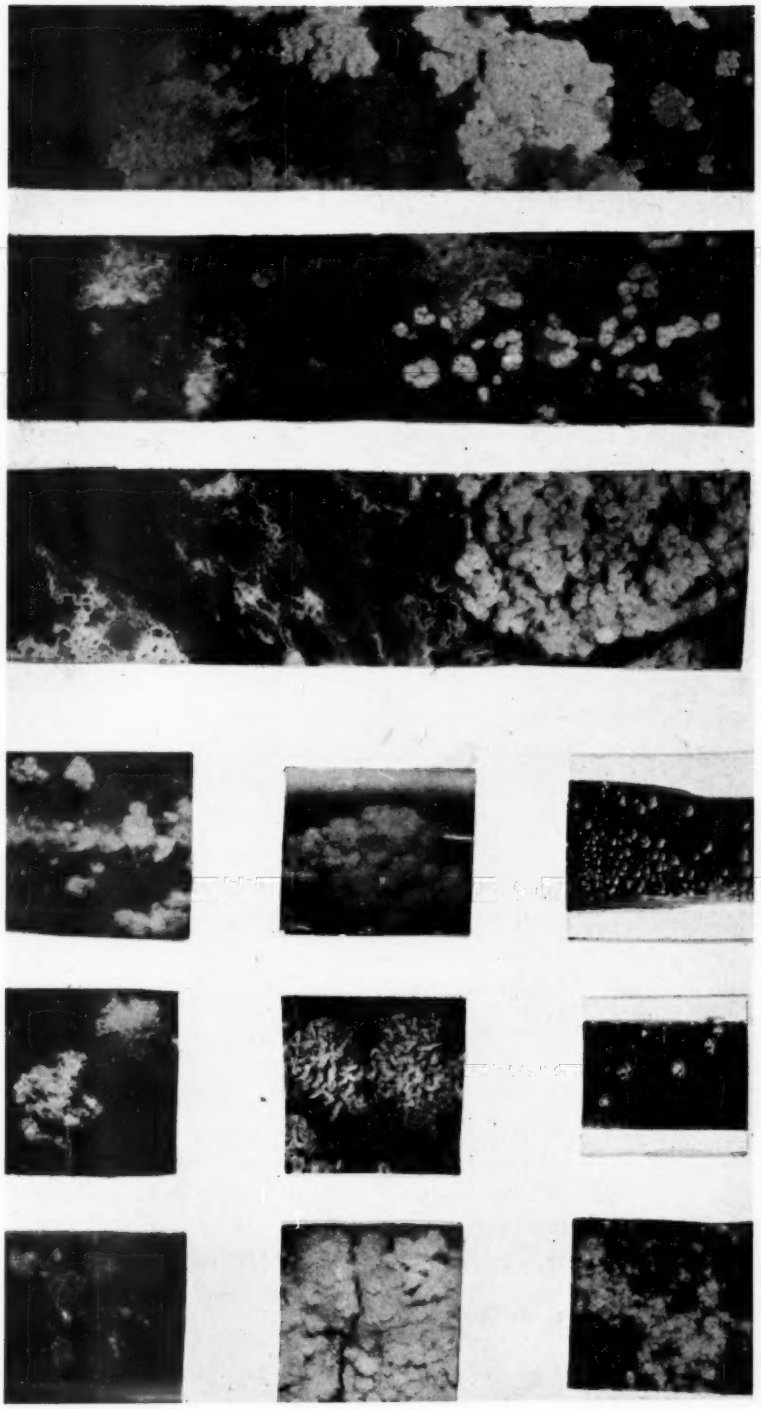

PLATE 2

to the condition of the patient or the ability of his serum to fix complement. Accordingly, 13 tuberculous sera were obtained through the kindness of Dr. D. Hauptman then at Seaview Hospital, S. I. These sera were divided into two classes; those from healing cases with good prognosis, and those from actively progressing cases with poor prognosis. There were 7 of the former and 6 of the latter. The controls consisted of 1 serum from a healthy adult showing a negative Mantoux reaction, 2 sera from children immunized with *BCG*, and 1 serum from an apparently healthy adult who gave a decidedly positive Mantoux reaction.

Ten per cent amounts of each serum were added to glycerine-potato agar used as a base for the Bordet-Gengou medium. An inoculum light enough to show individual colonies was seeded on the slants. Strains H3 and H37 were the human strains used in this experiment. Of the 17 sera used for testing their dissociating power, 2 only dissociated both H3 and H37 into round convex, glistening S colonies. Both of these sera gave negative complement-fixation reactions. One serum was from an improving tuberculous patient, and the other serum was from the adult who showed a positive Mantoux, although apparently nontuberculous in an active sense. The colonies obtained with the other sera were intermediate or R in character. It is possible that second, third or fourth generations of these bacilli on the same sera would subsequently have shown induced dissociation. Not enough material was provided to test this out, as only 10 cc. of serum or blood were given by each person.

Complement-fixation tests were performed on these sera. The antigens used for the complement-fixation tests were freshly made up saline suspensions of H3, *BCG*, and avian tubercle bacilli in 0.5 per cent phenol. The other reagents were kindly supplied by Dr. Annis Thomson of the Bureau of Laboratories of the New York City Department of Health. They had been titrated for complement-fixation tests for gonococcus infection. The general technique followed was that described in the 1933 edition of Park and Williams's *Pathogenic Microörganisms*.

Four of 6 active cases, and 3 of 7 healing cases showed fixation. The controls, including 3 additional sera from tuberculin-negative children which proved insufficient for dissociation tests, were negative.

The fact that dissociation from R to S was obtained with only one tuberculous serum, and this one showing no fixation of complement whereas 6 other sera did show fixation, indicates that ability to fix complement does not bear any relation to the dissociating ability of a

serum. No definite conclusions can be drawn at present. Nevertheless, the results obtained with these few sera suggest that the serum of a tuberculous patient does not necessarily show marked ability to dissociate the human tubercle bacillus, and moreover that the serum of a person not actively tuberculous may show marked ability to dissociate.

Attempt to Dissociate Human Strains with Sera Obtained from Rabbits Immunized with H37 Protein Fraction M.A.100

Two rabbits were immunized against H37 protein fraction M.A.100 obtained from the H. K. Mulford Company. This protein was derived from cultures of H37 human tubercle bacilli, and the chemical fractionation was carried out by Johnson and Anderson at Yale University under the auspices of the National Tuberculosis Association.

The purpose of this experiment was to determine whether or not anti-tuberculoprotein would be able to bring about the dissociation of human tubercle bacilli. Ten doses of 10 mgm. each of protein were given intravenously. The rabbits were given a rest period of several weeks, and were then bled with aseptic precaution from the heart. Ten per cent amounts of these sera were added to glycerine-potato-agar, and seeded with strains H37 and H3. Two normal rabbit sera and Bordet-Gengou medium served as controls. The control rabbit sera all fixed complement, but to a lesser degree than the immunized animals. The dissociating power of the anti-tuberculoproteins was not great, since intermediates of the Rs and RS type were obtained, but no true S. The normal rabbit sera gave flat wrinkled colonies of an Rs type.

The Relation of R and S to Virulence

The relative virulence of R and S organisms derived from the same strain, and the relative virulence of dissociants derived from different strains has been studied. In carrying out the virulence tests the following factors were taken into consideration:

1: Possible individual variation in resistance by the animals
2: Size of the dose
3: Type of lesion produced—diffuse or limited, macroscopic or microscopic, calcification and fibrous tissue
4: Degree of illness shown, and general condition of the body at autopsy—emaciated or well nourished
5: Dissociation in the body as shown by cultures

6: The possible existence and multiplication of the tubercle bacillus in the body in a non-acid-fast form
7: The presence of other organisms

The type of disease produced by S dissociants differed markedly from that produced by R bacilli. The picture at autopsy of animals which had received S was as follows: The bodies were usually emaciated, and the lymph nodes much enlarged and filled with pus. The livers were very much enlarged, soft, and haemorrhagic, but seldom showed macroscopic lesions. This was also true of the kidneys. The spleens were enormously enlarged, and either studded with tiny pearl-like lesions or else with large areas of yellowish-white caseation. The lungs in the case of the mice had marked congestion, and in the case of guinea pigs inoculated intraperitoneally with the human type S, numerous round grayish glassy lesions scattered throughout the lung.

In animals which had received R organisms, the lymph nodes were usually calcified, hard, knotty, and with little or no pus. The liver, spleen, and sometimes kidneys showed macroscopic lesions, but less enlargement and inflammation. The work of Sabin and her colleagues (1931), both with whole living avian tubercle bacilli and with chemical fractions, indicates that R stimulates the production of walled-off tissue to a much greater extent than S, unless the size of the dose be so overwhelming that the body cannot keep pace with the multiplication of the organisms.

Undissociated human tubercle bacilli from Bordet-Gengou medium of the SR or Sr type, inoculated intraperitoneally, seemed to give a mixed picture. The virulence was almost equal to that of the true S, but there were more macroscopic lesions. There were large caseous areas in the spleen, and the liver was enlarged and haemorrhagic. All told, the S and Sr bacilli of one strain were more virulent than R bacilli of that *same strain.* However, the R organisms of one strain may be more virulent than the S organisms of another strain. This is apparently the case with the H3 and Arilotta strains, the R of the H3 being more virulent than the S of the Arilotta.

Reed and Rice (1931) report that recently isolated virulent cultures, and other cultures with a history of virulence produce S colonies, whereas less virulent or avirulent cultures give R. They studied 28 strains of avian, bovine, and human tubercle bacilli. This does not appear to be the case with the few strains studied and reported here. The human strain Arilotta gave from the very first isolation a more S-like type of

colony than the H3. It has, however, proved considerably less virulent than the H3 strain. Smithburn, in his very recent paper states that the S type is by no means always virulent.

The autopsy findings on the various animals is given in the subsequent tables. No lesion was considered tuberculous unless it showed tubercle bacilli on smears stained with Ziehl-Neelsen's carbolfuchsin. The possibility of the existence of non-acid-fast forms of the tubercle bacillus in lesions was considered. Many of the smears suggested very strongly the presence of granule forms. These forms, which will be taken up in greater detail in a future report, may correspond to the well known "G" forms found by Hadley and others in studies on the dissociation of other organisms, such as the gonococcus.

Smears made from severe lesions showed, in some cases, many acid-fast bacilli, in other cases few or none at all. This was especially noticeable in the Arilotta strain. Coccoid non-acid-fast "G" forms resembling forms observed on smears made from pure cultures, were often seen on smears in which few or no acid fast organisms were found. They were also present on some of the smears on which acid-fast rods were abundant. These coccoid forms appear in tuberculous sputum.

Since most of the Arilotta R guinea pigs died of other infections, their shorter survival was not considered significant. The guinea pig which received 5 mgm. of S, however, survived 87 days longer than did the guinea pig which received 5 mgm. of R. The latter showed no sign of other infection.

The guinea pig, which received 5 mgm. of undissociated Arilotta, was outlived by the R guinea pig 27 days. Moreover, the guinea pig which received 0.1 mgm. of S of the H3 strain survived 28 days longer than the one which received only 0.01 mgm., and 98 days longer than the one which received 0.1 mgm. of undissociated H3. These differences in survival time which seem contrary to the general trend suggest the possibility of considerable variation in resistance among individual guinea pigs. On the whole, however, the gross pathological findings indicate that S tubercle bacilli produce severer and more extensive lesions than the R bacilli of the same strain. When the dose is beyond a certain limit (as in the case of those pigs which received 5 mgm.), it would appear that the body is equally overwhelmed by R, as it cannot encapsulate the enormous number of organisms fast enough to limit their spread to any considerable degree.

The fact that in several instances not complicated by the presence of

TABLE 10

STRAIN	ANIMAL	DOSE (MGM.)	SURVIVAL	SEVERITY OF LESIONS	SMEARS
Avian R, old Board of Health Strain	rooster	0.5 intraperitoneally	5½ mos. (killed)	none found	no acid-fasts found
Avian S old Board of Health Strain	rooster	0.5 intraperitoneally	4 mos. (died)	diarrhoea loss weight liver enlarged, haemorrhagic no macroscopic lesions	no acid-fasts found: "g" forms
Avian R, Rockefeller Institute	rooster	0.5 intraperitoneally	3½ mos. (killed in fight)	10–12 large encapsulated yellow lesions in liver	liver ++++ spleen +
Avian S, Rockefeller Institute	rooster	0.5 intraperitoneally	3½ mos. (killed)	diarrhoea loss weight liver enlarged, haemorrhagic, one white lesion	liver ++++ "g" forms testis acid-fast coccoid forms
Avian R, Rockefeller Institute	mouse	0.5 intraperitoneally	4 mos. (killed)	8–10 large lesions in liver large peritoneal and intestinal lesions lesions all of encapsulated type	organs ++++
Avian R, Rockefeller Institute	mouse	0.5 intraperitoneally	5 mos. (killed)	lesions throughout; some encapsulated; some diffuse	organs ++++
Avian R, Rockefeller Institute	mouse	1.0 intraperitoneally	4½ mos. (killed in dying condition)	lesions throughout, some encapsulated, some diffuse	organs ++++
Avian S, Rockefeller Institute	mouse	0.5 intraperitoneally	1 mo. (died) *M. tetragenes* in culture of peritoneum	no visible lesions; liver haemorrhagic	liver ++ intestine ++ peritoneum "g" forms
Avian S, Rockefeller Institute	mouse	0.5 intraperitoneally	4 mos. (died) *M. tetragenes* recovered from heart's	emaciated: lesions in liver, peritoneum, intestines, lungs congested	organs ++
Avian S, Rockefeller Institute	mouse	1.0 intraperitoneally	4½ mos. (killed)	ill, not emaciated liver enlarged, haemor-	liver +, many "g" forms spleen ++++ intestine

Avian S, Rockefeller Institute	mouse	1.0 intraperitoneally	4½ mos. (killed)	ill, not emaciated liver enlarged, haemorrhagic, pulpy one gross lesion in spleen	liver +, many "g" forms spleen ++++ intestine ++++ lungs, kidney "g" forms heart's blood +
H37R from Long's medium	Guinea pig 107	1.0 subcutaneously	22 days (died) pneumonia gram + cocci	inguinals enlarged lungs congested	inguinals +
H37R from Long's medium	Guinea pig 71	1.0 subcutaneously	4 weeks (killed)	lymph nodes enlarged	lymph nodes + peritoneum +
H37 "membrane" (Sr or SR) from Long's medium	guinea pig 1472	1.0 subcutaneously	4 weeks (died)	inguinals retroperitoneal lymph nodes enlarged	lymph nodes +++
H37 "membrane" (Sr or SR) from Long's medium	guinea pig 78	1.0 subcutaneously	3 weeks (died) pneumonia, gram-positive cocci	inguinals retroperitoneal lymph nodes enlarged lungs congested	lymph nodes +++ gram-positive cocci
H37R from Bordet-Gengou plate after animal passage	guinea pig 301	1.0 subcutaneously	8 weeks (killed)	inguinals retroperitoneal lymph nodes enlarged	lymph nodes + spleen +
H37R from Bordet-Gengou plate after animal passage	guinea pig 302	1.0 subcutaneously	3 weeks (died) pneumonia, gram-positive cocci	inguinals retroperitoneal lymph nodes slightly enlarged lungs congested	no acid-fast bacteria found
H37S from Bordet-Gengou plate after animal passage	guinea pig 303	1.0 subcutaneously	2 weeks (died) pneumonia, gram-negative rods	inguinals slightly enlarged	no acid-fasts found
H37S from Bordet-Gengou plate after animal passage	guinea pig 304	1.0 subcutaneously	8 weeks (died)	emaciated inguinals retroperitoneal much enlarged, thick pus	lymph nodes ++++ liver, spleen, "g" forms

TABLE 11

Virulence tests on guinea pigs with H3 and Arilotta strains

STRAIN	DOSE (MGM.) (INTRAPERITONEALLY)	S			R			UNDISSOCIATED (Sr)		
		Survival (days)	Severity of lesions	Smears	Survival (days)	Severity of lesions	Smears	Survival (days)	Severity of lesions	Smears
H3 (human)	5	29	very severe	+++ "g" in liver, spleen, kidney, peritoneum	33	severe abscess site inoculation	+++ "g" in kidney	33	very severe	+++ "g" in spleen, peritoneum
	1	24	very severe	+++ "g" in liver, peritoneum	105	severe	+ in lymph node	35	very severe	+++ "g" in kidney
	0.1	133	very severe	+++	126	severe	++ in lungs; "g" in liver	35	very severe	+++
	0.01	105	findings obliterated by p.m. changes		123	severe	+ few acid-fasts	105	severe	+ many "g" in spleen
Arilotta (human)	5	165	findings obliterated by gas bacteria		78	severe	+++ many "g" in kidney	105	findings obliterated by gas bacteria	
	1	273 (killed)	severe	lymph nodes + "g" in lungs	190	moderate	++ other infection; "g" in lungs	6	negative	− other infection
	0.1	123	severe	lymph nodes + many "g"	155	moderate	+ other infection; "g" in lungs	281 (killed)	moderate	− "g" in liver, lungs
	0.01	273 (killed)	severe	+ lung and lymph nodes, "g" in liver	185	moderate	+ in lymph nodes, other infection; "g" in lungs	281 (killed)	moderate	+ in lymph nodes, lungs; "g" in liver, lungs

Abbreviations and symbols: +++, many acid-fast bacilli; + few acid-fasts; ++ moderate number; − no acid-fasts found.
"g" means granule forms.

other infection, S guinea pigs survived even longer than the R guinea pigs suggests that besides virulence there is another factor in the relationship of the tubercle bacillus to the guinea pig. This factor is the relative ability of R and S to stimulate protective antibodies. It would seem that the S while producing more extensive lesions, on the other hand, stimulates greater immunity as well. This is in agreement with the finding of specific antigens present in S which are not found in R, and applies not only to the tubercle bacillus but to other organisms as well, for example the pneumococcus. The comparative value of S and R as vaccines cannot be stated with certainty until the subject has been more extensively investigated. The protective value of H3 and Arilotta S organisms is now being studied.

Discussion: an Interpretation of the Mechanism Underlying Dissociation

Serological studies with various bacteria, cited by Hadley in his comprehensive monograph *Microbic Dissociation*, indicate that the S form carries a double antigen, while the R form usually contains a simple antigen. Reed and Rice (1931) have reached a similar conclusion from their careful studies of R and S tubercle-bacillus antigens by means of complement-fixation. Antisera prepared from R organisms seemed to lack S-specific antibody, but contained a higher proportion of group antibodies.

How can we explain these facts? How can we explain the fact that each bacillus usually possesses the potentiality of becoming either an R or an S form; that immune sera, small traces (in optimal concentrations) of specific chemicals in the medium, pH, moisture, and other environmental conditions, are able to promote one type, and partially or completely suppress the other? How can the apparent stability of cultures such as the *BCG* be accounted for?

The only mechanism which seems able to explain all of these facts is a catalyst mechanism. It seems possible that R and S, and their accompanying characteristics such as virulence and antigenic content, represent response to environmental conditions by specific bacterial catalysts, one of which catalyzes the formation of substances leading to S, and the other of which catalyzes the formation of substances leading to R. There also could conceivably be but a single catalyst, which under some conditions could produce S-specific substance, and under other less favorable conditions was partially inhibited or modified so that intermediate or R forms resulted. In catalyst terminology, the

Abbreviations and symbols: +++, many acid-fast bacilli; + few acid-fasts; ++ moderate number; — no acid-fasts found. "g" means granule forms

formation of S tubercle-bacillus substance or substances may be promoted by blood and additional small amounts of ferric chloride; or that catalytic process may be wholly or partially suppressed by lack of blood or iron, or in the case of Long's medium and probably to some extent in Petroff's medium, by the presence of small amounts of crystal- or gentian-violet. In an apparently analogous way, enzymes and inorganic catalysts are promoted or "poisoned." HCN, 1/10,000 normal, prevents iron in blood charcoal from oxidizing amino-acids, and also poisons the iron-containing respiration ferment in living cells, the dye congo-red, at a molar concentration of 1/1,000,000,000, will destroy 90 per cent of the activity of the enzyme fumarase. There are innumerable examples of this sort in both biology and chemistry.

It may not be going too far afield to mention a few experiments with iron which have recently appeared in biological literature. Iron strip added to beet-juice medium caused a marked increase in the growth of an organism responsible for a disease of beet plants (Cameron, 1934). Iron, in an optimal concentration of 0.4 per cent, was found to act as a catalyst for the establishment of the oxidation-reduction potential of alloxantin (Hill and Michaelis, 1934). Crile (1932) reported that oxygen is consumed and CO_2 given out by so called "autosynthetic cells." These are cell-like structures consisting of mixtures of protein and lipoid extracts of tissue. According to Crile, an R.Q. of 0.82 was obtained, and proteins were utilized. This metabolism-like phenomenon could be increased or decreased and arrested by various agents. Dilute ferric chloride increased the oxygen consumption from 20 to 70 times. Maximum effects were obtained with concentrations of 0.0001 n – 0.001 n or 0.0054 – 0.054 grams/litre. $FeCl_3$ also increased the cataphoretic charge on freshly prepared "cells" (pH 7.4). The maximum increase was at a concentration of 0.0001 mol or 0.0162 grams/litre. The maximum effect of $FeCl_3$ for dissociating human tubercle bacilli from R to S was found to be with 0.004 grams/litre. Kahn and Scharzkopf (1931) found that S avian and bovine tubercle bacilli showed somewhat greater cataphoretic speed than the R organisms of the same strains. Ferric chloride which favors S formation in Bordet-Gengou medium may also be a factor in increasing the cataphoretic charge and velocity. There is, however, much careful work still to be done before any definite correlations of this sort can be established.

Ferric chloride, 0.25 per cent, given intravenously, is claimed by Menken (1934) to considerably increase the span of life in experimental

tuberculous rabbits. In relatively high concentrations, and in the presence of other environmental factors, the action of ferric chloride may be of a different nature.

In an experiment on rats carried on at the U. S. Bureau of Fisheries, the animals were fed organic arsenic as found in shrimp, and approximately the same quantity of arsenic as arsenic trioxide. They found that the carcasses of rats which had received arsenic in shrimp contained about 4 times the quantity contained in the stock diet controls, but the rats which had received the inorganic arsenic contained from 55 to 65 times that in the controls. Thus iron, as found in blood haemoglobin, apparently may not exert the same influence, or at least to the same extent, as inorganic iron, upon the morphology and virulence of the tubercle bacillus, even though horse-haemoglobin contains as much as from 0.335 to 0.470 per cent of iron.

An interesting illustration of the effect of a mere trace of metal upon a biological mechanism in which enzymes most certainly play a major rôle, is that of the setting of the larval oyster. Dr. Herbert F. Prytherch, Director of the U. S. Fisheries Biological Station at Beaufort, North Carolina, has discovered that setting requires a definite chemical stimulus for its initiation, and that copper is the only known element that will induce the reaction. It has a marked effect under natural conditions in minute concentrations of one part copper to 2 to 10 million parts of sea water. The copper is taken into the digestive tract of the larval oyster in the form of colloidal copper oxychloride.

To return to the subject of dissociation, it seems reasonable to believe that, since every cell possesses the potentialities of becoming either R or S, the catalysts of R and S substances, if they exist, are probably directly or indirectly under the control of some gene or genes in the hereditary mechanism of the cell. Hadley has stated: "Behind microbic dissociation there must exist a biological mechanism, and this mechanism we shall see concerns microbic heredity." Surely the genes constitute the innermost citadel of the hereditary mechanism. Moreover, genes are not only self-duplicating catalysts (Alexander and Bridges, 1928), but direct most other cell activities. Since dissociation is usually reversible, and sensitive to environment, we cannot consider a dissociated bacterium a mutant. It does seem reasonable to consider the *dissociant* as a form in which the genes or other cell catalysts responsible for R and S formation, have been temporarily affected due to the presence or absence of specific substances in the milieu. The possibility of *tem-*

porary gene or other catalyst modification has been suggested by J. Alexander (1931, 1934).

If a dissociant fails to reacquire its former characteristics, even after a number of generations' growth on media ordinarily favoring such changes, it would seem that an irreversible change had taken place involving some gene. One might then be justified in calling such an organism a mutant. *BCG* has been found nonvirulent after many generations of growth on media supposed to favor increase in virulence (Park and Mishulow, 1931; Mishulow, Park, and Melman, 1932). Moreover the pH curve of *BCG* on Long's medium was distinctly of the human rather than the bovine type. Possibly the *BCG* strain has become irreversibly altered, although this matter is hotly disputed.

More experimentation and study will perhaps greatly alter our present conceptions of the mechanism which underlies dissociation. The catalyst interpretation affords a satisfactory explanation for the results obtained thus far by the writer and others. It is presented here as a hypothesis which may, at least, prove useful in stimulating further work.

SUMMARY AND CONCLUSIONS

1. Three strains of human tubercle bacilli have been completely dissociated from R to S.

2. Human type S colonies are convex, round, smooth-edged, and glistening, and resemble the S colonies of bovine and avian tubercle bacilli.

3. The addition of small optimal amounts of ferric chloride, 0.0004 per cent, to Bordet-Gengou medium favored the formation of S colonies of the human type in pure culture.

4. Lack of sufficient blood in Bordet-Gengou medium, and the addition of crystal-violet to Long's medium are inhibitory to the formation of S colonies. Crystal-violet in 0.01 per cent amounts discouraged avian S, and a concentration of 0.001–0.0001 per cent sufficed to inhibit the human-type S.

5. The lesions produced by avian and by human R bacilli tended to be limited, calcified, and macroscopic, and contained relatively few bacilli, whereas those produced by the S organisms were more diffuse, inflammatory, small or microscopic, and contained relatively more bacilli.

6. The S tubercle bacilli of a given strain tended to cause more symptoms of illness and emaciation, and were somewhat more virulent than R organisms *of the same strain*. In the case of two strains which differed

in virulence, the R of one strain was more virulent than the S of the other strain.

7. One out of 13 sera taken from tuberculosis patients was able to completely dissociate the human tubercle bacillus, strains H3 and H37, from R to S in one generation under described conditions. The serum of a nontuberculous person, showing a positive Mantoux reaction, was able to dissociate these human strains completely in one generation.

8. The ability of a tuberculosis patient's serum to fix complement did not appear to bear any relation to the dissociating power of his serum.

9. The sera of two rabbits immunized against H37 tubercle-bacillus-protein fraction M.A.100 did not dissociate strains H3 and H37.

10. A catalyst mechanism has been suggested as the possible mechanism underlying the dissociation phenomenon.

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TENTATIVE COMPREHENSIVE DIAGNOSTIC STANDARDS FOR TUBERCULOSIS

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The tenth edition of *Diagnostic Standards* issued in 1935 by the National Tuberculosis Association is restricted to a consideration of the diagnosis, treatment and classification of tuberculosis of the lungs and tracheobronchial lymph nodes. Through the medium of this brochure the National Association is rendering an invaluable service, not only in broadcasting the knowledge that primary and reinfection types of intrathoracic tuberculosis are separate and distinct clinical conditions and that the changed reactive capacities induced in tissues by an antecedent infection have a profound influence on the behavior of subsequent infections, but also in partially standardizing terminology whereby the designation "Childhood Type of Tuberculosis" officially replaces a confusing assortment of names formerly applied to the first-infection type of intrathoracic tuberculosis.

Doubtless, for excellent reasons, *Diagnostic Standards* recommends no terminology applicable in general either to all varieties of tuberculosis of the first-infection type or to all forms of tuberculosis of the superinfection or reinfection variety, and suggests no detailed plan for subclassifying tuberculosis patients. A comprehensive consideration of the diagnosis and classification of tuberculosis provides an opportunity to present a panoramic concept of the disease which conceivably may prove useful. With these and other objectives in view, an attempt is made here to outline a general orientation of tuberculosis. Obviously, a satisfactory performance of this task transcends individual ability, thus at best this treatise can merely perform temporary functions while the development and adoption of an official *Comprehensive Diagnostic Standards*, embodying the combined opinion of a group interested in tuberculosis, is awaited.

THE CLASSIFICATION OF TUBERCULOSIS

A slight modification of the descriptive method found satisfactory and practical for classifying all tuberculosis patients studied at the

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Lymanhurst Health Center in Minneapolis during the past fourteen years may be outlined briefly as follows:

COMPREHENSIVE PLAN FOR CLASSIFYING TUBERCULOSIS

I: First-Infection Type of Tuberculosis (Primary Tuberculosis)
- *A:* Nondemonstrable by X-ray
- *B:* Demonstrable by X-ray
  - *1:*[2]*Primary intrathoracic tuberculosis (childhood type according to *Diagnostic Standards*)
  - *2:* Primary intraäbdominal tuberculosis
  - *3:* Primary tuberculosis of cervical region
  - *4:* Primary tuberculosis of other parts of the body

II: Superinfection or Reinfection Type of Tuberculosis (Secondary Tuberculosis)
- *A:* Superinfection or reinfection type of pulmonary tuberculosis
  - *1:*[2]†Phthisis (the adult type according to *Diagnostic Standards*—minimal; moderately advanced; far advanced
  - *2:* Tuberculous pneumonia
  - *3:* Miliary tuberculosis
- *B:* Tuberculosis of other viscera
- *C:* Bone-and-joint tuberculosis
- *D:* Tuberculosis of serous membranes
- *E:* Tuberculosis of skin
- *F:* Miscellaneous reinfection types of tuberculosis

This classification is based primarily on the premise that two general types of tuberculosis occur in man, namely, the "First-Infection Type" and the "Superinfection or Reinfection Type," each of which owes its existence as well as its peculiarly distinctive characteristics to the presence in the tissues at the time an infection is laid down, either of normal preinfection or of abnormal postinfection immunological properties respectively. The "First-Infection Type of Tuberculosis" probably is analogous to Ranke's "Stage I"; whereas the "Reinfection Type of Tuberculosis" doubtless is equivalent to Ranke's "Stages II and III" combined.

Apparently functional and structural characteristics peculiar to each organ and tissue are primarily responsible for the introduction of diversity into the general clinical picture of each of these two general types of tuberculosis, and, to meet this situation, provision is made in the Lymanhurst Classification for dividing patients with primary or second-

[2] *–† Diagnostic standards is restricted to a consideration of these two conditions.

ary tuberculosis into subgroups in accordance with which viscus or tissue is involved.

The important fundamental similarities common to all patients with primary tuberculosis exclusively place great restrictions on the significance of subclassifying these individuals into separate and distinct groups on the basis of X-ray examinations which under ideal conditions usually reveal only a part of the pathological processes present. Doubtlessly group distinctions dependent upon X-ray findings are apt to be inaccurate and should be resorted to mainly for descriptive purposes, and preferably without permitting this procedure to distort the broad view that each subgroup represents an artificial subdivision of one general type of tuberculosis designated here by the name, "First-Infection Type of Tuberculosis."

In the Lymanhurst classification the disease produced by a first infection of the lungs and tracheobronchial lymph nodes, which obviously is only one of many anatomical subvarieties of primary tuberculosis, is designated for several reasons by the term "Primary Intrathoracic Tuberculosis" rather than by the name "Childhood Type of Tuberculosis" employed in *Diagnostic Standards*. The Lymanhurst designation synonymous with the name "Childhood Type of Tuberculosis" fits conveniently into a comprehensive and systematic plan for classifying all forms of tuberculosis of the first-infection type; the term "Primary Intrathoracic Tuberculosis" indicates clearly that the disease is considered to be the result of a first infection with tubercle bacilli and that some of the resultant pathological charges are known on the basis of X-ray studies to be situated in the thorax; and last, the Lymanhurst term avoids an implication inherent in the name "Childhood Type of Tuberculosis" to the effect that children are peculiarly susceptible to the first-infection type of intrathoracic tuberculosis. Since chance extrinsic factors rather than specific intrinsic conditions related to age operate to time the occurrence of exogenous first infections, an avoidance of implications that suggest the existence at various periods in life of significant differences in susceptibility to primary contaminations seems desirable.

Apparently the term "Childhood Type of Tuberculosis" does not fit conveniently into a comprehensive and systematic plan for classifying all primary forms of the disease, and adherence to this name leads to awkward situations when an attempt is made to select logically related names suitable for primary tuberculosis situated in regions other than the thorax. The advisability, therefore, of abandoning the name

"Childhood Type of Tuberculosis" in favor of an equally appropriate but more adaptable term probably merits consideration.

At the time the present official name for primary tuberculosis of the lungs and tracheobronchial lymph nodes was adopted, need existed not only for reducing a chaotic terminology to a uniform standard, but also for focusing attention on the view that primary and reinfection forms of intrathoracic tuberculosis are separate and distinct clinical conditions. The inclusive terms "First-Infection" and "Reinfection Type of Tuberculosis" used in the Lymanhurst classification seem to satisfy these needs, and simultaneously perform the additional functions of serving as general headings under which all cases of tuberculosis can be descriptively subclassified in a systematic manner and of fashioning a unifying orientation whereby special anatomical varieties of primary or secondary tuberculosis are listed as component units of one or the other of two general types of the disease.

The policy of subgrouping all secondary forms of tuberculosis under one general heading as carried out in the Lymanhurst classification seems justified for the following reasons: (1) the clinical characteristics of all secondary forms of tuberculosis have features in common which are different from those typical of primary varieties of tuberculosis; (2) the reinfection forms of the disease are responsible for most of the disability and appreciable illness as well as for practically all of the deaths due to tuberculosis and usually require special therapy; (3) they often display a tendency to cause progressive tissue necrosis to a marked degree; (4) and lastly these conditions are the direct product not of first infections but of endogenous or exogenous reinfections deposited in allergic tissues.

Although patients with tuberculosis of the reinfection variety also have primary tuberculous lesions somewhere in their bodies, nevertheless, to classify these cases in the reinfection group exclusively seems practical, due to the fact that the secondary form of the disease which they have developed postdating their primary infection is of major importance and overwhelmingly dominates the situation in so far as health, life and need for therapy are concerned.

EVOLUTION OF TUBERCULOSIS

(*The First-Infection Type*)

A first infection with tubercle bacilli initiates the development of a series of interrelated phenomena which make their appearance in a

very orderly sequence. The phenomena which collectively form the picture of primary tuberculosis probably never fail to materialize after a first infection occurs, whereas the changes that comprise the picture of secondary tuberculosis may or may not develop. The approximate order of the stages through which tuberculosis seems to pass as its complete evolution unfurls may be outlined briefly as follows:

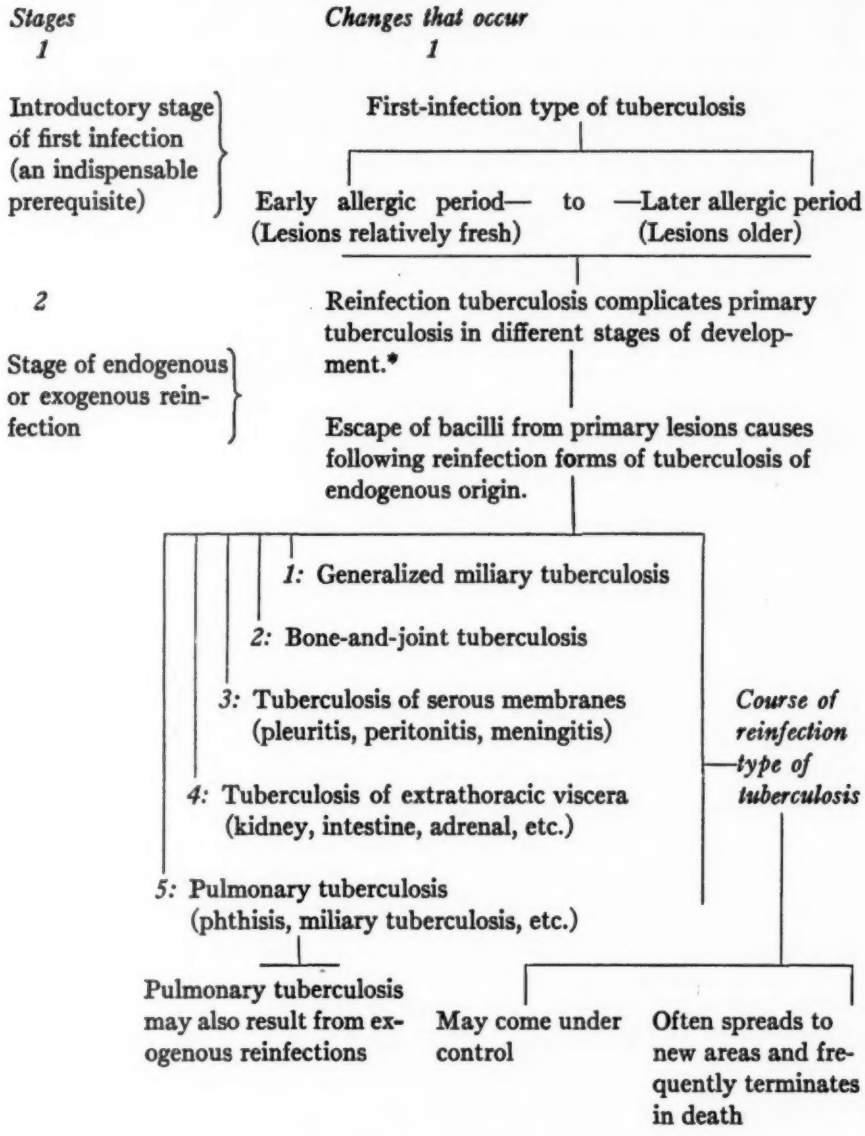
TUBERCULOSIS

1. Evolution of The First-Infection Type of Tuberculosis

Stages	*Changes that occur*
1	*1*
Symptomless pre-allergic stage. (3–8 weeks' duration.)	First infection followed promptly by a dissemination of bacilli. Neutrophils phagocytose and focalize bacilli at multiple points. Monocytes appear, surround and phagocytose neutrophils and bacilli. Monocytes are converted into epithelioid cells (the tubercle).
2	*2*
Early allergic stage. (2 years' duration.)	Fever, tuberculin sensitivity*, transitory symptoms, caseation appear. Lesions may now be visible on films. Lesions remain grossly stationary for months. Finally, a slow resolution starts, which leads to
3	*3*
Late allergic stage. (Many years' duration.)	Fibrosis or calcification. Ossification and revascularization of primary lesions may occur. Virulent bacilli persist in lesions for years.
4	*4*
Healed stage.	Death of bacilli and disappearance of tuberculin sensitivity mark the complete cure of primary tuberculosis. This may not occur during the span of human life.

Note: Other complex immunological changes accompany the development of tuberculin sensitivity.

2. Evolution of the Reinfection Type of Tuberculosis


Stages
1
Changes that occur
1
Introductory stage of first infection (an indispensable prerequisite)
First-infection type of tuberculosis
Early allergic period— to —Later allergic period
(Lesions relatively fresh) (Lesions older)
2
Reinfection tuberculosis complicates primary tuberculosis in different stages of development.*
Stage of endogenous or exogenous reinfection
Escape of bacilli from primary lesions causes following reinfection forms of tuberculosis of endogenous origin.
1: Generalized miliary tuberculosis
2: Bone-and-joint tuberculosis
3: Tuberculosis of serous membranes (pleuritis, peritonitis, meningitis)
4: Tuberculosis of extrathoracic viscera (kidney, intestine, adrenal, etc.)
5: Pulmonary tuberculosis (phthisis, miliary tuberculosis, etc.)
Course of reinfection type of tuberculosis
Pulmonary tuberculosis may also result from exogenous reinfections
May come under control
Often spreads to new areas and frequently terminates in death

*The reinfection type of tuberculosis may complicate the first-infection type shortly after it is laid down or months and years later.

As is indicated in this outline, the first-infection type of tuberculosis is considered to be an introductory prerequisite indispensable for the development of the reinfection type of tuberculosis. An antecedent infection followed later by the appearance of secondary tuberculosis, therefore, is pictured as the sequence of the events that lead to the development of the reinfection type of the disease, and subsequent extensions of the disease are considered to be equivalent to repetitions of the second stage of this process. The biphasic character of the evolution of tuberculosis as well as the occurrence of two general types of the disease in man are contingent on the parallel existence in the population of two distinct immunological states on the part of the host, by virtue of which the tubercle bacillus produces the relatively benign primary type of tuberculosis when it lodges in normal tissues but may cause a more serious secondary form of the disease when it invades tissues whose immunological properties have been specifically and complexly altered by an antecedent infection.

DEFINITION FOR FIRST-INFECTION AND REINFECTION TYPES OF TUBERCULOSIS

The "First-Infection Type of Tuberculosis" is defined as the disease produced by an initial infection of any part of the body with tubercle bacilli, and the "Reinfection Type of Tuberculosis" is defined as the disease produced in any part of the body by a reinfection with tubercle bacilli laid down in patients whose bodies harbor foci of primary tuberculosis and whose tissues were rendered immunologically abnormal by a first infection. The term initial infection is used here as applying to single or multiple implantations of virulent organisms on tissues before their normal immunological state has been specifically altered; whereas the term reinfection is employed as applying to single or multiple implantations of bacilli on tissues after their immunological properties have been modified by an antecedent infection. In the sense these terms are used, therefore, the development of the complex changes which accompany the appearance of tuberculin sensitivity are accepted as forming the dividing line that separates primary from reinfections.

Primary intrathoracic, intraäbdominal, cervical, etc., are special anatomical subvarieties of the first-infection type of the disease, whereas phthisis, miliary tuberculosis, tuberculosis of serous membranes, bone-and-joint tuberculosis, etc., are anatomical subvarieties of the reinfection type of tuberculosis.

TYPES OF TUBERCULOSIS OF DIFFERENT AGES

The first-infection type of tuberculosis may develop at any time during the span of life. In the past, this form of the disease has been discovered principally in children, but in the future, as increasing numbers pass puberty uncontaminated, the so-called "Childhood Type of Tuberculosis" may be found to occur as often in adults as it does in children if not more so. In all probability, environmental factors, independent of age or race, determine the time at which the incidence of new first infections is highest; and if this assumption is correct the assertions made in *Diagnostic Standards* to the effect that "the childhood type of tuberculosis is usually found in children but is not uncommon in adult Negroes, Mexicans, American Indians, Puerto Ricans and Filipinos" seem to need clarification. The hygienic condition under which these races live presumably should operate to permit relatively few candidates for primary tuberculosis to attain adult age uncontaminated and consequently one should expect the childhood type of the disease of recent origin to occur rather rarely in the adults of these races.

The lesions of primary tuberculosis are found most frequently in the thorax, but in districts where raw milk is used and tuberculosis is prevalent in dairy herds, the incidence of primary involvement of other regions of the body is quite appreciable. Regardless of where the disease is located, however, the pathological processes present in the lymph-node component of the primary complex usually exceed those present at the site of primary invasion. The lymph-node system of the body, therefore, is the great common repository for the initial infective dose of tubercle bacilli and its progeny.

Since the reinfection type of tuberculosis may complicate primary tuberculosis in any of its many successive stages of development, secondary varieties of the disease occur during all decades of life. Miliary tuberculosis, tuberculous meningitis, and bone-and-joint tuberculosis frequently appear during infancy and childhood, whereas phthisis displays a tendency not to develop until during or after the age of puberty. As a general rule lymph-node involvement does not occur in secondary tuberculosis to the degree it does in primary tuberculosis.

CHARACTERISTICS OF FIRST-INFECTION AND REINFECTION TYPES OF TUBERCULOSIS CONTRASTED

A: First-infection type	*B: Reinfection type*
1: A benign and rarely fatal condition, produced by a first infection deposited in tissue with normal immunological properties	*1:* A serious and often fatal condition produced by a reinfection deposited in tissues with abnormal immunological properties induced by an antecedent infection
2: Lesions usually resolve to leave trivial fibrosed or calcified deposits	*2:* Lesions resolve less consistently. May become fibrotic but usually do not calcify
3: Tendency of lesions to cause tissue necrosis is very limited as well as nonprogressive	*3:* Tendency of lesions to cause progressive tissue necrosis is quite marked
4: Lymph nodes are grossly involved as a rule	*4:* Lymph nodes are not grossly involved as a rule
5: Symptoms usually are absent, trivial or overlooked. If present they usually are transitory	*5:* Symptoms are often conspicuous, and tend frequently to increase in severity
6: Therapy is seldom required	*6:* Therapy is often required
7: Therapy does not modify the course of primary tuberculosis to an appreciable degree	*7:* Therapy often does modify the course of reinfection tuberculosis to a very appreciable degree
8: Illness, disability and death-rates are relatively low	*8:* Illness, disability and death-rates are relatively high
9: Primary tuberculosis is a relatively unimportant public-health problem	*9:* Reinfection tuberculosis (especially of the lungs) is a relatively important health problem
10: Institutions are of very limited value in the treatment of primary tuberculosis	*10:* Institutions are of great value in the treatment of secondary tuberculosis
11: The clinical subvarieties recognized are: Primary intrathoracic Primary intraabdominal Primary cervical and Primary tuberculosis of other regions of the body	*11:* The clinical subvarieties recognized are reinfection tuberculosis of the lungs; of various other viscera; of serous membranes, of bones and joints, etc.

DIAGNOSIS OF TUBERCULOSIS

A positive cutaneous reaction to tuberculoprotein establishes a diagnosis of tuberculosis of the first-infection type regardless of whether the patient is young or old and irrespective of the presence or absence during life of symptoms or of demonstrable lesions. The demonstration of tuberculin sensitivity in a young child or in an adult known recently to be nonallergic are circumstantial factors which in either instance permit deductions that the primary disease is of recent origin. A positive test yields no information relative to the location, extent, or stage of development of the primary pathological process, and it also fails to disclose the presence of tuberculosis of the reinfection type. The intensity of the reaction, however, may roughly parallel the degree to which the normal immunological state of the host has been generally modified.

In the absence of acute illness, a negative response to adequate doses of properly administered tuberculin provides very dependable evidence that no tuberculosis of any variety is present.

X-ray examinations contribute an important share to the diagnosis of tuberculosis by disclosing the gross appearance, extent, location and progress of roentgenographically demonstrable lesions. This method of study is indispensable particularly for the detection of phthisis in its minimal stage as well as for excluding secondary pulmonary tuberculosis in tuberculin sensitive patients. Shadows and rarefactions cast on the film by pathological changes due to various causes, however, often resemble one another; thus errors attend expert film interpretations quite frequently. Positive X-ray findings, therefore, do not obviate the necessity of making other examinations of diagnostic significance.

The presence of tubercle bacilli in the sputum establishes a diagnosis of tuberculosis, but fails to indicate which form of the disease (primary or secondary) is present. Sputum examinations are also of public-health significance. Since infants and young children do not expectorate, pharyngeal swabs or gastrointestinal contents must be examined if a search for tubercle bacilli is indicated.

Other laboratory tests, together with the physical examination, history, symptoms, temperature curve, weight, etc., also contribute important evidence of value, but to a lesser extent probably than is true of the tuberculin test, the X-ray study and the sputum examination. Special manifestations indicative of involvement of special organs or

parts of the body are quite important and always demand careful investigation.

In general a diagnosis of tuberculosis carried to the point of correct differentiation as to its type is based on the assembled evidence derived from several sources, each of which contributes important but limited bits of information.

TREATMENT

Apparently no therapeutic measures are known to man that modify the course of tuberculosis of the first infection type to an appreciable degree. The treatment of this condition is limited, therefore, to bed-rest while symptoms are present and to the observance at all times of general hygienic and dietetic measures, such as promote optimum health and nutrition. The possibilities as well as the need for treating primary tuberculosis are so limited that little opportunity remains for preventoria, Summer Camps and Open Window Schools, to provide measures of benefit that cannot be obtained equally well, and far more economically in tuberculosis free private homes.

Tuberculosis is a dangerous communicable disease, thus prophylactic measures designed to protect patients from continued and repeated infections are imperative, and, if neglected, an otherwise ideal regimen is doomed to result in many failures.

The value of active special therapy of tuberculosis is limited essentially to its use in combating reinfection forms of the disease. In general, the basic principle of the therapeutic measures that prove exceptionally beneficial involves the direct application of rest to the area of disease. Also various types of pulmonary collapse therapy are of public health importance in that they operate to prevent contagion by rendering the sputum free of tubercle bacilli. Reinfection forms of tuberculosis can be treated successfully in homes, in private hospitals, or in sanatoria. Competency on the part of the attending physician is of major importance, whereas the type of institution in which therapy is administered is of minor importance.

CONTROL OF TUBERCULOSIS

In order to be successful and complete, a program of tuberculosis eradication must be directed toward the elimination of two sources of contagion which are mainly responsible for the perpetuation of tuberculosis in the human race.

The bovine source has been reduced to a position of minor importance throughout most of the United States not only by destroying bacilli present in dairy products by pasteurization; but also by eradicating tuberculous cattle from herds. By completely eliminating bovine tuberculosis from counties and states, the veterinarians of America have accomplished one of the most extensive practical demonstrations of communicable disease control the world has witnessed.

Progress in the prevention of tuberculosis of human origin has lagged behind that made in preventing bovine tuberculosis. However, without additional scientific information or methods, the medical profession is now in a position to eradicate tuberculosis derived from human sources, merely by applying methods modeled somewhat after the plan already proved successful by the veterinarians.

Since pulmonary tuberculosis of the reinfection type is the form of the disease that is mainly responsible for the transfer of tuberculosis from person to person, the detection of this form of the disease is of primary importance in a program for eradicating human sources of contagion. An ideal program for accomplishing this goal demands the application of tuberculin tests to the entire population, followed by chest films of all positive tuberculin reactors at regular intervals. These steps lead to the identification of potential or active spreaders of the disease. In addition provision must be made either in homes or institutions for isolating all patients found with pulmonary tuberculosis of the reinfection type and germ-laden sputum, and the period of isolation should not be terminated until the patient no longer discharges bacilli.

A program of this type of national scope can be launched at the present time provided physicians certify all families under their care as free from communicable forms of tuberculosis, by routinely applying tuberculin tests followed by chest plates of all positive reactors.

If inadequate finances do not permit an ideal program, a modified plan which involves routine tuberculin testing with subsequent X-ray studies, particularly of the adult tuberculin-sensitive members of families in which children are found to have positive reactions to tuberculoprotein, is of value. In this economical program X-ray studies of the adult positive reactors are not insisted upon if the children in the family are found not to be infected. This modified plan of family certification may be improved upon at a relatively little expense, by making fluoroscopic examinations of the infected members of the group.

The removal of disseminators of tubercle bacilli (patients with phthisis

particularly) from families and from all professional and nonprofessional groups, if carried to completion, should give us a population well certified as free from communicable forms of the disease, and the present decline in the tuberculosis death-rate in America should then be greatly accelerated.

Interesting experiments are now being conducted which involve the administration of attenuated tubercle bacilli (*BCG* particularly), for the purpose of inducing an active immunity to tuberculosis. Final judgment as to the value of these procedures probably should be deferred pending a study of the results obtained over a period of several years. The production, however, of an active immunity to the disease probably will fail to produce beneficial results on a par with those known to accrue from measures designed to prevent tuberculous infections.

CONCLUSIONS

Doubtless, by broadcasting a concise scientific orienting interpretation of tuberculosis through the medium of an *Official Comprehensive Diagnostic Standards*, a uniform and standardized attack can be focused on the disease, in which all participants in the effort to eradicate and to treat tuberculosis are familiar with the views and recommendations of a group of experts. This tentative venture possibly indicates the general lines along which an acceptable official brochure can be constructed.

The sources from which the views presented here were derived, together with more detailed discussions of tuberculosis, are contained in references 1 to 15 inclusive.

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A STUDY OF THE INCIDENCE OF TUBERCULOSIS IN STATE INSTITUTIONS IN MINNESOTA[1]

HERBERT A. BURNS[2]

This report contains the results of the investigation of tuberculosis among inmates and employees of institutions under the charge of the Minnesota State Board of Control. The work was carried on by the staff of the Minnesota State Sanatorium. Intracutaneous (Mantoux) testing was started September 1, 1934. One member of the staff, detailed to this work, remained constantly in the field giving the test and reading the reactions. Old Tuberculin, diluted 1/1000, was used. Following this, the positive reactors were X-rayed. Paper films were decided upon with arrangements that, in case of necessity or doubt, celluloid films might be used upon request. The X-ray work was done in the institutions by approved technicians. All plates were sent to the State Sanatorium for reading and recording. The clerical work entailed was carried on by the sanatorium staff.

For the purposes of convenience the institutions have been so arranged that group A refers to inmates of insane hospitals and asylums, group B to inmates of schools for the feeble-minded and epileptic, group C to those of penal institutions, and group D to those of schools and orphanages.

The Minnesota State Board of Control, responsible for the care of state wards, interested itself in the survey to determine the incidence of tuberculous infection and disease as a preliminary step in the development of a logical control program. The investigation of tuberculosis, of both infection and disease, was carried out for the purpose of identifying those infected at the present, those showing definite evidence of parenchymal disease, and those who show the presence of tubercle bacilli in their sputum. It was, therefore, hoped that this survey would furnish information upon which the Board of Control might build its future tuberculosis program in Minnesota.

[1] Read at a meeting of the Minnesota State Hospital Association, Lutsen, Minnesota, June 21, 1935.

[2] Minnesota State Sanatorium, Ah-gwah-ching, Minnesota.

INSANE HOSPITALS AND ASYLUMS, FEEBLE-MINDED AND EPILEPTICS

From the study of tuberculosis deaths in the state institutions during the six-year period prior to the survey, information was obtained relative to the introduction of tuberculosis into the institutions as well as its spread among the inmates. Deaths of 528 patients occurred in 8 institutions caring for the insane, feeble-minded and epileptics during the period from January 1, 1929 to December 31, 1934, as follows:

YEAR	PATIENT POPULATION	TOTAL DEATHS IN INSTITUTIONS		PER CENT OF DEATHS DUE TO TUBERCULOSIS	TUBERCULOSIS CASES DIAGNOSED
		General	Tuberculosis		
1929	9,937	724	97	13.39	103
1930	10,159	760	82	10.79	102
1931	10,606	683	74	10.83	101
1932	11,127	714	86	12.04	120
1933	11,674	745	94	12.67	176
1934	11,901	883	95	10.75	219

Because of the intimate association in institutional life, exposure from unknown and unisolated positive-sputum cases must necessarily be greater than would occur from such cases in the average community. Such close association exists among all of the 15,994 inmates and 2,400 employees. Visiting of friends and relatives, as well as parole cases leaving the institutions for home, increases the number of individuals exposed through contact beyond those associated with the case in the institution.

If the institution is a reservoir of infection, the many thousands of visitors associating with unknown inmate-cases each year only add to the necessity of protective measures being carried out. It would appear, from the incidence of tuberculosis as well as the deaths from this disease in state institutions, that these dependents form one of the reservoirs of infection in the state which should be corrected as a part of the state's program of tuberculosis eradication among its citizens.

To determine a possible relationship between the reported deaths and the existing cases, these deaths during the past six years were studied. Five hundred and thirty-nine deaths were tabulated according to their employment in the institution. Four hundred and sixty-three did no work. Fourteen did occupational therapy; seven worked in the laundry; forty-three were occupied in ward work; one did tailoring; six worked

on the farm; one in the kitchen, three in the dining room, and one in the bakery.

The total duration of known tuberculous illness occurring among inmates of different age-groups and at variable periods of time following commitment suggested the cause for the increased-infection rate as well as case-rate among this group. Thirty-three per cent of the deaths occurred one year or less following the admission of the individuals concerned. Thirty-six per cent of those who died within this period were 45 years of age or over. Thus, it would appear that tuberculosis is frequently brought into the institution through new commitments.

Fifty-one per cent of the deaths among inmates 25 years of age or over occurred among those who were committed five years or more before clinical tuberculosis first became manifest. This group represents chronic unrecognized cases bringing the disease with them from the outside, and the new cases developing as a result of contact with the unknown cases in the institution.

The study of the death-reports points to the fact that protection of the inmate body in the future can be accomplished to a large extent by diagnosing the cases before or immediately following commitment, and providing the necessary isolation quarters for clinical cases to the end that their association with the uninfected can be prevented.

It is apparent that the problem developed from two main roots: the introduction of cases through commitments, and the development of new cases through exposure in the institution. The protection of the institution against the tuberculosis patient's admission can be easily accomplished through the Mantoux testing of all admissions and X-raying of all positive reactors, including both inmates and employees. Protection against the cases developing after admission requires a medical service whose major duty it is to diagnose such cases throughout the period of the individual's residence. This entails a very complicated administrative set-up which might be simplified by considering tuberculosis in this group as a major problem. This would require a separate administrative set-up.

Tuberculosis inmates should be segregated from those who show no evidence of tuberculosis other than the positive skin reaction. This group is again divided into two groups: those who have evidence of old parenchymal infiltration on X-ray, and those who show, in addition, the presence of tubercle bacilli in the sputum. The former group must be examined periodically for additional signs of disease and for the

TABLE 1

Deaths in State institutions in relation to length of residence

AGE	TOTAL DEATHS	TIME IN THE INSTITUTION PRIOR TO DEATH																											
		Months			Years																								
		−1	1-3	4-11	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	25+
−19	18	1		2	2	4	3	3	1		2																		
19-24	51	1	4	5	8	10	4	6	5	1	4	1		2															
25-29	69	2	3	8	9	7	7	2	6	1	6	7	7	1	1		1			1									
30-34	68		5	4	4	5	10	6	6	3	2	7	4	3	4	4								1					
35-39	58	3		4	4	6	8	8	2	4	4		1	3	1		5	3	1					1					
40-44	50	3	1	4	2	7	2	4	2	1	3	6	2	2	1	2		2	2	1	2	1							
45-49	47	2	4	2	2	2	2	3	2	5	3	2	4	1	2	3	1	1			1	1	1		1		1		1
50-54	39	5	5	1	3	5	1	3	1	1	1	2			1	2	1	2		1	1					1	1	1	
55-59	34	2	2	2		5	2	3		1	3		2	1	2		1		1		1		2			1	1	1	1
60-64	28	1	1	3		1		1		2	1	1	1		1	1	1	2	1	2	1		2	2				1	2
65-69	30	2	1	2	1	1	2			1		1	1	1	2		1		1	2	1	2		1	1	1			5
70-74	16	2					1	2					2			1			1	1				2				1	3
75-79	16				1		1				1	1	2	1			1				1				1				2	4
80-84	6			1																					2			1		2
85+	9								1	1															1	1		1		4
Total..	539	24	26	38	36	53	43	41	26	21	30	28	26	15	15	13	12	10	7	8	8	4	5	7	6	3	3	5	4	22

presence of tubercle bacilli in the sputum. Such cases, when found, must be included in the positive-sputum group. There must be repeated X-raying of this segregated negative sputum group in order to find promptly the individual who is breaking down with clinical, as well as symptomatic, tuberculosis. It is essential also that the positive-sputum cases be identified promptly in order that effective isolation may be established without delay.

The vital problem in handling the tuberculous involves the positive-sputum case. Such a case, developing among the inmates of the state schools and penal institutions, may be very easily handled, since their numbers are few and the facilities for their immediate isolation are adequate. The need for added isolation facilities for the positive-sputum cases developing among the inmates of the state hospitals, asylums and schools for the feeble-minded and epileptics constitutes a very serious problem, not only because of the number of such cases, but also because of their mental condition. This class will require absolute isolation because of their inability to coöperate, which makes contagious technique difficult as a part of an administrative unit in a domiciliary institution. To make it possible to use a reasonably careful technique, it will be necessary to add trained attendants in order that the sputum and other discharges from this group of patients can be completely collected and promptly destroyed. In order to control tuberculosis in the state hospitals at the present time, facilities for the isolation of 230 patients showing tubercle bacilli in their sputum are necessary. It is also necessary to provide efficient segregation for 1,073 inmates whose tuberculin reactions were positive and whose X-rays showed definite evidence of parenchymal involvement.

The relationship between infection as shown by the positive skin reaction and the development of parenchymal infiltration as revealed by the X-ray, as well as in the sputum, has been studied. Among the 11,517 inmates in the state hospitals and asylums and schools for the feeble-minded and epileptics, 82 per cent reacted positively to the tuberculin test. Of the total group 9.2 per cent showed evidence of parenchymal infiltration on X-ray, while 11.2 per cent of the positive reactors showed evidence of pulmonary disease. Tubercle bacilli were demonstrated in 1.9 per cent of the inmates. These positive sputum inmates represent 2.4 per cent of those reacting positively to the skin test, and 21.3 per cent of those whose X-ray gave evidence of parenchymal infiltration. Assuming that the death-rate will be constant in the future

INCIDENCE OF TUBERCULOSIS INFECTION AS REVEALED BY MANTOUX TESTING OF INMATES AND EMPLOYEES IN STATE INSTITUTIONS::
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
1196 Mantoux Tested
996 Positive Reactors
674 Positive X-rays
150 Showing Parenchymal Infiltration on X-ray
53 Positive Sputum Cases Identified
Anoka State Asylum
1074 Mantoux Tested
1031 Positive Mantoux Reactors
671 Positive X-rays
132 Showing Parenchymal Infiltration on X-ray
8 Positive Sputum Cases Identified
Hastings State Asylum
1402 Mantoux Tested
1380Positive Mantoux Reactors
895 Positive X-rays
168 Showing Parenchymal Infiltration on X-ray
34 Positive Sputum Cases Identified
Willmar State Asylum
1832 Mantoux Tested
1595 Positive Mantoux Reactors
1027 Positive X-rays
167 Showing Parenchymal Infiltration on X-ray
29 Positive Sputum Cases Identified
Fergus Falls State Hospital
1539 Mantoux Tested
1039 Positive Mantoux Reactors
702 Positive X-rays
93 Showing Parenchymal Infiltration on X-ray
24 Positive Sputum Cases Identified
Rochester State Hospital
1926 Mantoux Tested
1786 Positive Mantoux Reactors
1278Positive X-rays
245 Showing Parenchymal Infiltration on X-ray
58 Positive Sputum Cases Identified
St. Peter State Hospital
1989 Mantoux Tested
1387 Positive Mantoux Reactors
990 Positive X-rays
92 Showing Parenchymal Infiltration on X-ray
28 Positive Sputum Cases Identified
School For Feeble Minded
554 Mantoux Tested
552 Positive Mantoux Reactors
393 Positive X-rays
26 Showing Parenchymal Infiltration on X-ray
15 Positive Sputum Cases Identified
Colony For Epileptics
2430 Mantoux Tested
1747 Positive Mantoux Reactors
1359 Positive X-rays
91 Showing Parenchymal Infiltration on X-ray
16 Positive Sputum Cases Identified
Employees

CHART 1

as it has been during the past six years as shown above, 0.75 per cent of the total inmates will die of tuberculosis during the next year; 0.92 per cent of the positive reactors to the Mantoux test, 8.2 per cent of the individuals with parenchymal disease and 38.4 per cent of those with positive sputum will likewise die of tuberculosis during the next 12-month period.

Table 5 gives, in detail, the bacteriological study of sputum and stools examined from both inmates and employees. Because of the large number of deteriorated individuals in the state hospitals and asylums who were unable to give specimens of sputum, it was decided to collect both sputa and stools uniformly.

The hydrocarbon flotation as well as the direct-smear method was used in searching for tubercle bacilli. Three negative specimens of sputum were obtained before the case was considered a noncarrier.

Of the total cases 8.8 per cent were found to have tubercle bacilli in the stool specimen, while only 2.4 per cent of the total cases were found positive on direct-smear examination of sputum. The highest incidence of positive findings occurred in the flotation examination of sputum. By this test, 13.4 per cent of all cases of tuberculosis in the state hospitals and asylums showed tubercle bacilli.

EMPLOYEES

Under conditions similar to those carried out in the examination of inmates, 2,430 employees were examined. Of the total employees, 72 per cent reacted positively to the tuberculin test. Of the total employees, 3.7 per cent were found on X-ray to have parenchymal tuberculosis. This group represents 5.2 per cent of the positive reactor group of employees. Of the total employees 0.66 per cent were found to have tubercle bacilli present in their sputum. These positive-sputum employees represent 0.92 per cent of the positive reactors and 1.7 per cent of the parenchymal cases found among employees.

SUMMARY

Many problems concerning the epidemiological control of tuberculosis were brought out as a result of the work entailed in this survey. Several questions involving the administrative control of the disease become important in any complete program applicable to these institutions.

It is apparent that institutions in which people live for a prolonged period of time may become more widely infected with tuberculosis

than would occur in the community from which the individual was originally committed. Unless definite control measures are taken, such institutions may become reservoirs of infection. One of the chief sources of infection, in the institutions studied, has been the home neighborhood, since it is evident that a large proportion of the cases admitted came into the institution without a diagnosis of tuberculosis and without an opportunity for the institution to protect itself against the exposure incidental to the inmate's entrance.

CONCLUSIONS

The ideal administrative program would include the tuberculin testing of all individuals entering institutional life, either as inmate or employee, followed by the X-raying of all positive reactors. Those already a part of the institution, who show evidence of parenchymal involvement with negative sputum, should be segregated and reëxamined at stated intervals in order that reactivated, or missed cases of positive sputum may be found before much exposure has occurred. This part of the institutional control work can well be cared for by the present personnel, except for the Mantoux testing and the reading of the X-ray plates, which should be done continuously under the auspices of the Director of the Division of Tuberculosis, State Board of Control.

The most serious public-health problem involved relates to the positive-sputum case. At the present time 243 such cases have been found as a result of this survey. Others will be found by reëxamining the segregated negative-sputum inmates and still others in the future will be found during the process of commitment. Adequate handling of this group of inmates determines the success of any control program applicable to state institutions. The protection of the institutions against the committed insane who also suffer from tuberculosis has not been given due consideration. As a part of all such commitments, a tuberculin test and X-ray should be considered obligatory. With this as a part of the routine, all negative- and positive-sputum pulmonary tuberculosis cases would be identified and segregated or isolated before associations in the institutions could be established.

The care of the positive-sputum cases should not be the responsibility of a domiciliary institution. The communicable disease problem superimposed upon the mental problem results in the former becoming of paramount importance. The isolation of the tuberculous inmates in an institution separate from the one specializing in the domiciliary care

of the mental case should be provided, in order that contagious technique can be given first place in the management of the sick, and in order that the public-health problem involved may not be overlooked in the institutional routine. For this reason facilities should be provided making it possible for the institution to remove from its jurisdiction positive-sputum cases, accepting their return as soon as they have convalesced to a point where there is no longer a public health problem involved.

To prevent the employment of tuberculous individuals, tuberculin testing and X-raying of positive reactors must be made a routine procedure incidental to employment. Employees must also be examined periodically in the future by routine Mantoux testing and X-raying of positive reactors.

TABLE 2

Incidence of tuberculous infection and disease according to age-group among inmates and employees

AGE GROUP	INMATES			EMPLOYEES			INMATES AND EMPLOYEES	
	Positive Mantoux	Negative Mantoux	Per cent Positive	Positive Mantoux	Negative Mantoux	Per cent Positive	Second Infection Tuberculosis	Per cent Positive*
0–1	1	22	4%					
1–4	3	42	7%					
5–9	29	216	12%					
10–14	198	571	26%				7	3.2%
15–19	475	848	36%	4	7	36%	19	3.8%
20–24	923	680	58%	211	190	53%	51	4.5%
25–29	1,007	310	76%	209	120	64%	78	7.0%
30–34	1,049	224	82%	241	77	76%	99	7.7%
35–39	1,087	158	87%	223	60	79%	104	7.9%
40–44	1,229	150	89%	200	60	77%	156	10.9%
45–49	1,313	137	90%	184	35	84%	140	9.4%
50–54	1,118	157	87%	157	47	77%	121	9.5%
55–59	905	116	86%	132	33	80%	116	11.2%
60–64	738	167	81%	71	24	75%	93	11.5%
65–69	553	92	85%	55	12	82%	85	14.0%
70–74	402	116	77%	18	6	75%	64	15.2%
75–79	234	60	79%	4		100%	39	16.4%
80 plus	155	56	73%				33	21.3%
Not given	90	20	82%	28	12	76%	14	11.8%
Total.....	11,509	4,142	74%	1,747	683	72%	1,219	9.2%

* Per cent of positive reactors with second-infection tuberculosis.

TABLE 3

Tabulation by institution of Mantoux tests and X-ray examinations

Inmates

GROUP	MANTOUX POSITIVE		MANTOUX NEGATIVE		NUMBER NOT X-RAYED		NUMBER X-RAYS TAKEN		X-RAY NEGATIVE		X-RAY CALCIFIED LYMPH NODE		X-RAY PRIMARY NODES		X-RAY PLEURISY		SECOND INFECTION TUBERCULOSIS	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Group A	4,362	3,265	669	673	40	26	4,322	3,240	1,549	1,151	2,180	1,596	144	95	160	117	515	440
Group B	908	1,031	164	445	18	19	891	1,012	298	275	520	659	16	26	23	21	58	60
Group C	1,640	94	911	301		2	1,640	92	725	38	826	52	67	4	73	1	51	1
Group D	141	68	689	290	1	1	151	76	20	16	129	59	3	1	1		2	1
Total inmates	7,051	4,458	2,433	1,709	59	48	7,004	4,420	2,592	1,480	3,655	2,366	230	126	257	139	626	502
Employees																		
Total employees	971	776	294	389	9	33	968	743	271	185	630	511	38	30	41	18	50	41
Grand total	8,022	5,234	2,727	2,098	68	81	7,972	5,163	2,863	1,665	4,285	2,877	268	156	298	157	676	543

TABLE 4

Tabulation of Mantoux tests and X-ray examinations of all institutional inmates according to age

AGE-GROUP	POSITIVE MANTOUX		NEGATIVE MANTOUX		NUMBER NOT X-RAYED		NUMBER OF X-RAYS		X-RAY NEGATIVE		X-RAY CALCIFIED LYMPH NODES		X-RAY PRIMARY NODE		X-RAY PLEURISY		SECOND INFECTION TUBERCULOSIS	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0–1	1		11	11			1		1									
1–4	1	2	13	29		1	1	1	1	1								
5–9	14	15	115	101			14	16	8	7	6	9	1					
10–14	130	68	339	232	2	1	131	71	44	16	80	54	2	2	1		6	1
15–19	244	231	442	406	1		249	235	69	67	173	152	3	5	5	3	6	13
20–24	642	281	548	132	4	10	640	271	195	93	411	152	9	7	21	4	22	22
25–29	748	259	239	71	5	6	743	253	286	101	400	129	15	3	21	2	46	20
30–34	718	331	121	103	3	9	715	322	319	108	334	172	24	10	17	6	54	38
35–39	644	443	79	79	5	3	639	440	274	146	308	241	17	7	15	8	49	46
40–44	708	521	64	86	5	4	703	517	254	148	365	294	25	14	22	18	75	64
45–49	755	558	52	85	6	5	749	553	295	191	361	297	32	18	20	25	82	51
50–54	682	436	74	83	4	2	678	434	261	156	335	224	28	18	34	15	66	48
55–59	528	377	56	60	5	1	523	376	189	133	272	189	18	9	26	12	51	54
60–64	396	342	96	71	5	2	391	340	129	107	202	184	15	14	26	17	46	43
65–69	330	223	44	48	4	1	327	222	129	74	155	103	17	5	11	14	38	41
70–74	231	171	63	53	3		228	171	65	67	116	75	12	5	19	10	38	25
75–79	131	103	31	29	5	3	126	100	36	36	63	45	4	5	8	1	20	18
80+	83	72	32	24	2		81	72	21	18	33	35	4	3	9	3	18	15
Not given	65	25	14	6			65	26	16	11	41	11	4	1	2	1	9	3
Total.....	7,051	4,458	2,433	1,709	59	48	7,004	4,420	2,592	1,480	3,655	2,366	230	126	257	139	626	502

TABLE 5

Tabulation of microscopic examinations done on all specimens from inmates and employees

Inmates

AGE-GROUP	NUMBER CASES BY X-RAY		NUMBER SPECIMENS RECEIVED				NEGATIVE SPUTUM						STOOL NEGATIVE		POSITIVE SPUTUM				STOOL POSITIVE		TOTAL POSITIVE	
			Sputum		Stool		1 Neg.		2 Neg.		3 Neg.				Smear		Flotations					
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
10–14	6	1	16	2	4	1					3		4				1	1		1	1	1
15–19	6	13	15	30	5	10				1	3	6	5	10		1	2	3			2	4
20–24	22	22	47	56	17	22					12	11	15	20	1	3	6	7	2	2	6	9
25–29	46	20	85	30	33	19		1	5		19	5	32	15	2		7	5	2	3	9	7
30–34	54	38	102	60	47	35			1	1	17	12	42	30	5	1	17	8	5	6	20	13
35–39	49	46	72	74	46	44	1	1	4	3	25	18	45	39	1	1	7	6	2	5	8	8
40–44	75	64	120	97	72	61	2	1	2	3	27	21	64	57		2	11	11	8	6	15	16
45–49	82	51	130	53	71	45	1	5	2	1	28	8	65	39	4	1	10	9	6	6	15	13
50–54	66	48	98	65	61	43	2	1	4	1	22	15	58	40	2	1	14	5	3	3	16	9
55–59	51	54	80	85	50	50	1		2	1	18	23	47	49		1	10	6	4	3	13	8
60–64	46	43	88	52	44	42	2	1	3	2	22	10	41	38		2	7	8	3	4	10	10
65–69	38	41	62	56	33	36	1		1	1	13	17	28	31	1		7	3	5	5	9	6
70–74	38	25	56	30	33	23		1	2	2	12	7	34	23	1		4	2	1	1	4	3
75–79	20	18	26	19	16	18		1			7	6	15	17			1		1		2	
80+	18	15	30	6	16	13	2	1	1		8	1	16	12			1	1		1	1	2
Not given	9	3	18	2	5	2	1				3		4	2	1		1	1			2	1
Total.....	626	502	1,045	717	553	464	13	13	27	16	239	160	515	422	18	13	106	76	42	46	133	110

Employees

Employees	50	41	94	71	23	23	3	1	5		20	15	22	22			8	8	1		8	8
Grand total.	676	543	1,139	788	576	487	16	14	32	16	259	175	537	444	18	13	114	84	43	46	141	118

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